

# Inquiry: The University of Arkansas Undergraduate Research Journal

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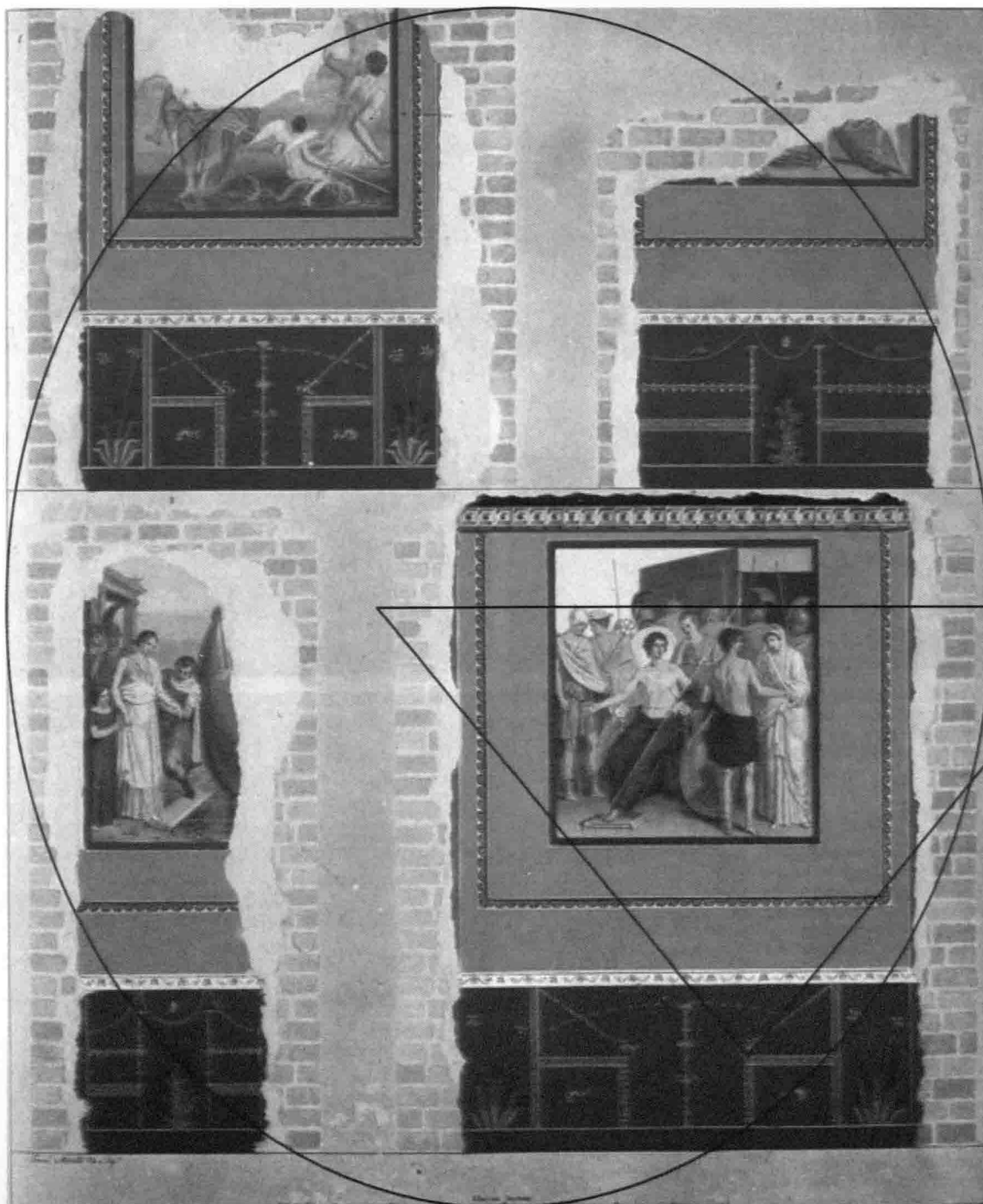
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# INQUIRY



Volume 10

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## ***INQUIRY: The Undergraduate Research Journal of the University of Arkansas***

### **Volume 10 – Fall 2009**

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## FOREWORD

The *Inquiry* journal is a project of the Teaching Academy of the University of Arkansas. The journal is supported financially and conceptually by the offices of the Provost and the Vice Provost for Research. Through print and on-line publication, *Inquiry* provides a forum for sharing the research and creative endeavors of undergraduate students at the U of A.

This print issue of *Inquiry* records the scholarly contributions of 14 U of A student/faculty mentor pairs during the 2008/2009 academic year. The full text is included for 13 manuscripts with an abstract for a 14th. Three manuscripts are also posted online on the *Inquiry* website (<http://inquiry.uark.edu/>). These manuscripts by Bollero, Thomas, and Tush received the Teaching Academy's Undergraduate Teaching Award. While the work by Bollero and Thomas could be reduced for print publication, the manuscript submitted by Tush was not as easily shortened.

As has been true in the past, the number of articles accepted for publication is controlled in part by the size of the journal, and we received many more high quality manuscripts than we could publish. The articles included in this issue are drawn from disciplines in five of the undergraduate colleges and schools at the University of Arkansas. They are representative of the quality of research done by students in the various disciplines represented on campus. The breadth of subject matter included here is testimony to the commitment made throughout the university to research at the undergraduate as well as the graduate level. Interdisciplinary work is featured in several papers.

Articles are selected for publication after review by faculty members. The range of disciplines at an institution like ours makes it impossible for a few individuals to evaluate critically all the papers submitted without the assistance of experts in specific disciplines. The Editor and Publication Board of *Inquiry* are grateful for the input of those campus colleagues who have served as reviewers. As Editor, I must also thank the members of the Publication Board who gave so generously of their time, particularly at the end of the spring semester when no one has any free time.

While the papers chosen for publication vary in subject, method, writing style, and manuscript format, they are uniformly excellent in content. As much as possible, we have endeavored to maintain discipline-specific styles to provide students with a publication experience comparable to one they might find in their individual disciplines. The intent of the journal is to record the depth and breadth of the scholarly activities of some of the university's best undergraduate students. I hope you enjoy the print and on-line offerings of the 2009 issue of *Inquiry*.

Barbara B. Shadden, Editor

## INQUIRY PUBLICATION BOARD, 2008-2009 ACADEMIC YEAR

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*The following individuals made the publication of Inquiry possible through their guidance and their willingness to take on reviewing responsibilities at the most hectic time of the academic year.*

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# THE EFFECT OF MUSICAL CHARACTERISTICS, EXPOSURE, AND INDIVIDUAL DIFFERENCE VARIABLES ON STRING STUDENT MUSICAL PREFERENCE: IMPLICATIONS FOR INTRODUCING WESTERN ART MUSIC

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## Abstract

*This study explores the influences of various musical, environmental, and personal factors on string students' preferences for selections of Western Art Music. The purpose of this study was to provide insight into the formation of music preferences by young string students in order to allow teachers to introduce Western Art music most effectively. Participants (n = 498) from northwest Arkansas public school string programs were given the String Student Music Preference Questionnaire (SSMPQ) developed by the author. Part One of the SSMPQ measured preference for six one-minute selections of Western Art music by Beethoven, Berlioz, Mahler, Saint-Saëns, and Schoenberg. In Part Two, the researcher collected data on participants' age, gender, musical experience, social influence and listening habits. It was determined that the musical examples with characteristics similar to popular music were most preferred. These characteristics included: fast tempo, steady rhythm, stable dynamics, identifiable instrumentation, and moderate complexity. Age, social influences, and listening habits did not significantly affect preference, while the gender and the live attendance portion of the musical experience variables significantly influenced participant preference.*

## Introduction

The introduction of Western Art music to beginning string students has become problematic due to the style's declining popularity. Starting in second grade, students may begin to show a preference toward popular styles (i.e., rock, pop, and rap) over Western Art music (Greer, Dorow, & Randall, 1974). In this study, Western Art music, often called "classical" music, refers to music of the Common Practice Period of Western musical composition. Preferences continue to center around popular styles and students may become less open to new music throughout junior high, until they reach adolescence in high school (Jin, 1999). It has even been suggested that junior high is the least opportune time to introduce new music to students due to their lack of open-mindedness about musical styles (Leblanc, Sims, Sivola, & Obert, 1996). However, most students begin instrumental study in junior high when they are required to encounter possibly unfamiliar Western Art music. The problem that arises, therefore, is how to introduce unfamiliar and unpopular music to students who are least open to experiencing and enjoying other musical styles.

There has been little research on beginning string students' music preferences. Most existing research has attempted to

define the relationships between music preference and musical characteristics (e.g., tempo), experience based variables (e.g., familiarity), or listener characteristics (e.g., age and social influences). Few have tied all of these factors together into a cohesive picture. Researchers often focus on the liking or disliking of single musical stimuli, which rarely presents a complete understanding of how preferences form and how teachers may influence preference formation in order to more effectively introduce new music. The purpose of this study was to explore string student musical preference through relationships between musical, exposure-related, and individual characteristics in order to give educators the knowledge and tools necessary to introduce Western Art music in a way that effectively generates student interest and lays a path for positive encounters with Western Art music in the future. More specifically, the current study was guided by three research questions:

1. What characteristics of Western Art music do sixth through twelfth grade strings students prefer?
2. How are student music preferences for each example influenced by fixed-factor variables?
3. What relationships, if any, exist between preference, interest, and familiarity?

The difference between the terms musical preference and musical taste as used in this study can be found in Price's (1986) glossary of terms dealing with affective response studies in music. Price defined preference as "an act of choosing, esteeming, or giving advantage to one thing over another" (p. 154). This contrasts with musical taste, which Price defines as "a person's overall attitude toward collective musical phenomena" (p. 154). In this study, preference is defined as a students' indicated liking for a single musical stimuli, whereas taste is the students' pre-formed attitude toward a larger body of music, such as the Western Art music genre.

## Literature Review

Previous music preference research can be divided into three primary categories. The first focuses on how musical characteristics, such as tempo or melody, influence preference. The music educator can easily manipulate musical characteristics in ways that positively influence students' musical choices. Leblanc, in multiple studies, found that faster tempi are clearly preferred over slower tempi (Leblanc, 1981;

Leblanc and McCrary, 1983). In addition, Montgomery (1988) found that faster perceived tempi are also preferred over tempi that sound slower. Students prefer rhythmic activity and clarity, along with pattern-based rhythms, according to Prince (1972). Fung (1996) found that dynamic variation and loud volume appeal to students. Music of moderate complexity, or music that is not overwhelmingly complex but also not too simple, appeals to students, according to Acevedo-Hernandez (2006). Students also prefer styles with instrumentations that are familiar (Jin, 1999).

The second type of research on music preference deals with exposure-related variables, such as familiarity and training. These variables are concerned with how often and in what environment the student encounters the selected music. Familiarity has a significant impact on preference, and students most often prefer music that sounds familiar (Getz, 1966; Hargreaves, 1984; Jin, 1999). Training, which can be viewed as a more in-depth extension of familiarity, can raise preference through rehearsals, guided listening, and other lessons (Droe, 2005; Baltagi, 2006). Teachers' attitudes toward the music they introduce also have an impact on preference and listening choice (Droe, 2005).

The third and final category of music preference research involves variables that are defined by the listener him or herself. These listener-related variables, such as musical training or age, may have profound effects on preference, but music educators often have little or no control over them. Rather than manipulating these variables, the educator can only consider them when introducing music to the student. Musical experience provides better vocabulary for listening to and describing musical preferences, according to Bundra (1993). Hargreaves, Comber, and Colley (1995) found that students with experience in ensembles that play less popular music, such as jazz or Western Art music, often show higher preferences for those genres. In general, students below 3rd grade are very open to different types of music, but above 3rd grade, students are much more resistant to new music (Greer, Dorow, & Randall, 1974). Openness is most limited in the junior high years (grades six through eight), though it does increase again in high school (Acevedo-Hernandez, 2006; Jin, 1999). Peer influence or teacher influence can have a strong effect on student preference, according to Alpert (1982) and Jin (1999). Females tend to have higher music preferences in general and higher preferences for Western Art music (Hargreaves, Comber, & Colley, 1995; Jin, 1999), along with higher preferences for music of slower tempi (Osborn, 1999).

## Methods

Students ( $n = 498$ ) in grades six through twelve from public schools in the Northwest Arkansas region participated in the study. This was essentially a convenience sample. Schools were selected based on proximity and the ability of the researcher to visit the teachers' classrooms at their convenience. All students were string players and a variety of teachers were used to determine if teaching style, or any

other variable influenced by the teacher, contributed to his or her students' listening habits. Student participation was based on the teacher's willingness to allow his or her classes to be surveyed. Students' participation was voluntary and no penalty was enacted against them for electing not to participate. IRB approval was granted on October 17, 2008.

## Instrument and Procedure

*The String Student Music Preference Questionnaire* (SSMPQ) was created for this study (see Appendix). Items were designed to measure student preferences for a variety of musical examples and to determine if these examples were related in any way to a variety of personal factors, such as musical experience or family and friends' musical preferences. Throughout the SSMPQ, the term "classical" was substituted for the term "Western Art" for ease of understanding, due to the age of the students being surveyed. Cronbach's Alpha was used to evaluate the reliability of the scaled response items on the SSMPQ. A calculated Cronbach's Alpha of .82 was considered highly reliable and robust enough for subsequent analysis.

Part One of the SSMPQ measured student preference for six different examples of Western Art music (see Appendix). Part One was divided into six identical sections, one for each musical example. For each section, students circled the number that most closely represented their preference for the example on a 1 (Dislike very much) to 5 (Like very much) Likert-type scale. Students indicated their familiarity with each example by indicating 'Yes' or 'No' when asked if they had ever heard the piece of music before. Students were also asked if the example made them want to listen to more classical music, which was also answered with 'Yes' or 'No'. This question was used to emphasize the students' overall preference for the example and explore if preference for a single example is related to desire to further explore classical music. The last question in each section of Part One, which was free response in nature, allowed students to supply any reasons why they did or did not enjoy the selection.

Six musical examples were chosen to examine student music preferences (see Table 1). *Beethoven Symphony V, Movement IV* was chosen due its quick tempo, likely unfamiliarity with young students, and moderate complexity. This dynamically stable excerpt includes clear melodies, predictable harmonic patterns, and motivic rhythms. Strings and brass are featured prominently.

*Beethoven Symphony VII, Movement II* was chosen due to its slow tempo, likely unfamiliarity with participants, and moderate complexity. It features clear melody and rhythm with a slight decrescendo to the conclusion of the example.

*Berlioz's Symphonie Fantastique, Witches Sabbath* was chosen due to the familiarity of the 'Dies Irae' theme, its fast tempo, loud dynamics, and moderate complexity. The very active harmonic movement and exciting figures in the strings make the excerpt sound fast and active, though the tempo is moderate.

Table 1. Characteristics of the examples on the SSMPQ.

	Tempo	Rhythm	Melody	Mood/ Modality	Harmony	Dynamics	Complexity	Instrumentation
Beethoven V	Fast	Stable	Simple	G Major	Predictable	Stable	Moderate	Full Symphony
Beethoven VII	Slow	Stable	Simple	A minor	Predictable	Stable	Moderate	Strings and Winds
Berlioz	Fast	Stable	Simple	A minor	Unpredictable	Loud then Soft	Moderate	Full Symphony with Brass featured
Mahler	Slow	Unstable	Simple	F Major	Unpredictable	Large Crescendo	Moderate	Strings and Harp
Saint-Saëns	Fast	Stable	Simple	G minor/Bb Major	Predictable	Stable	Low	Strings, Piano and Xylophone
Schoenberg	Slow	Unstable	Complex	Unclear	Unpredictable	Large Crescendo	High	Strings

*Mahler Symphony V Movement IV* was chosen for its slow tempo, moderate complexity, and unfamiliarity. The example begins quietly but crescendos continuously until the climax and cadence at the end. This example includes a clear but very slow melody and flexible rhythms that vary throughout.

*Saint-Saëns' Carnival of the Animals, Fossils* was chosen for its use of the familiar "Twinkle Twinkle Little Star" melody, its quick tempo, and relatively low complexity. This lighthearted example features the piano and xylophone as solo instruments, accompanied by rapid string movements, clear melodies, rhythmic motifs, and stable dynamics.

*Schoenberg's Verklärte Nacht, Movement IV* was chosen for its unfamiliarity, slow tempo, and high complexity. This excerpt features a smaller ensemble than the other examples and much less clear rhythm and melodic lines, along with large variations in dynamics.

Each example was numbered according to the previous list and the website <http://www.randomizer.org/form.htm> was used to generate random numbers 1 through 6. Three Compact Discs were copied with three different random orders to ensure that example order did not affect preference.

Part Two of the SSMPQ was concerned with other relevant personal information about the student that might influence his or her classical music preferences (see Appendix). The data collected from this section was compared with the students' preferences to see if any of these factors, which may or may not be manipulated by the educator, are important in forming each student's musical preferences. Questions probed current grade in school (since Greer, Dorow, & Randall (1974) found that age influenced students' preferences for classical music), gender, and string instrument currently being studied (violin, viola, cello, or bass) as well as additional instruments that they played. Students were also asked to indicate if they had studied privately and, if so, for how many years. Each respondent was then asked to list any other ensembles that he or she plays with. It may be possible that private lessons and extra-curricular performance habits contribute to musical experience and interest, which can be linked to preferences for classical music (Hargreaves, Comber, & Colley, 1995; Jin, 1999).

The next questions probed family members that play musical instruments, as well as minutes spent listening to popular and classical genres and whether or not they listened to classical music outside of school. The latter item was used to differentiate students who have a serious interest in the genre from those who rarely listen to classical music outside of their orchestra classroom. Students were also asked if their friends listen to classical music outside of school, since there may be a strong correlation between individual and peer preferences (Jin, 1999). The final questions probed whether anyone in the students' household listens to classical music (a factor, according to Jin, 1999) and whether they had ever attended a live performance of classical music, an experience that may influence student preference and interest.

The SSMPQ was given in the student's orchestra classrooms and each teacher agreed to allow the use of his or her class time for giving the SSMPQ. After a brief introduction and the statement that participation was not required, the researcher handed out the SSMPQ and ensured that each student had a writing utensil. Once the students were ready to take the survey, the first musical example was played. Students were asked to wait to fill in the questions regarding each example until that example had finished playing in order to ensure that the students' attention remained on the music rather than answering the questions. Students were given between 30 seconds and one minute to answer each section and were asked to put their pencils down upon completion of the section. The survey proceeded in this manner through each of the six examples. Each example was played only once. After completion of the listening section, students were asked to fill out the second part of the SSMPQ and then return their papers. Once all of the students had completed the SSMPQ, the researcher provided a quick word of thanks to the teacher and participants.

## Results

To answer research question 1, means and standard deviations were calculated. On the 5-point Likert-type scale (range 1-5, with 5 being "Like very much"), the most preferred example was Saint-Saëns' *Carnival of the Animals, Fossils* movement ( $M = 4.07$ ,  $SD = 1.073$ ) followed by Beethoven V, fourth movement, ( $M = 3.72$ ,  $SD = .985$ ), Beethoven VII, second movement ( $M = 3.62$ ,  $SD = 1.176$ ), Berlioz ( $M = 3.49$ ,  $SD = 1.114$ ), Schoenberg's *Verklärte Nacht*, fourth movement ( $M = 3.27$ ,  $SD = 1.221$ ), and Mahler V, fourth movement ( $M = 3.15$ ,  $SD = 1.112$ ). The participants also indicated their familiarity with each example. Students were most familiar with Beethoven VII (19.7% were familiar), followed by Beethoven V (13.9%), Schoenberg (13.9%), Saint-Saëns (13.5%), Berlioz (11.2%), Mahler (9.8%). Participants were asked if the example inspired them to listen to more music that is similar. Participants were most inspired to listen to music in the style of Saint-Saëns (65.9% answered Yes) followed by Beethoven V (56.0%), Beethoven VII (55.2%), Berlioz (51.6%), Schoenberg (46.2%), and Mahler (41.8%).

The characteristics shared by the most preferred examples (Saint-Saëns, Beethoven V, and Beethoven VII) include stable rhythm, simple melody, predictable harmony, stable dynamics, and low to moderate complexity. The Berlioz, Schoenberg, and Mahler examples were characterized by unpredictable harmony, large dynamic variations, and moderate to high complexity. Though tempo preferences were less clear due to the observation that Beethoven VII (slow) was ranked higher than Berlioz (fast), participants seemed to prefer excerpts with faster tempi. Instrumentation varied between each piece so patterns were difficult to distinguish. Mood/modality also varied greatly from piece to piece, so no patterns were evident. Figure 1 captures these outcomes schematically.

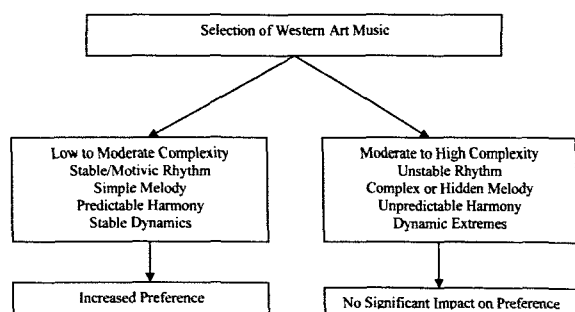


Figure 1: Model of the Impact of Musical Characteristics on Preference

In order to answer research question 2 and determine what variables may influence music preference for each of the six pieces selected for the SSMPQ, a series of one-way ANOVAs (Analysis of Variance) tests were conducted. A Bonferroni adjustment was made due to the number of ANOVA calculations conducted in the study in order to reduce the likelihood of Type I error. The alpha level used was .004, which was calculated by dividing alpha (.05) by the number of independent variables included in the test (14). Partial eta squared ( $\eta^2$ ) was used to determine the effect size for each variable in order to discuss practical as well as statistical significance. The preference for each example was compared with the following fourteen independent variables: grade (6-12), gender, instrument, number of additional instruments played, years of private lessons (0-9), number of other ensembles in which the student is involved, number of instrument players in the students' household, minutes of popular music listened to each day, number of minutes of Western Art music listened to each day, listening to Western Art music outside of school (Yes/No), friends listening to Western Art music, household members listening to Western Art music, attendance at a live performance, and teacher.

Participant preference for the Beethoven V, fourth movement excerpt was significantly ( $\alpha < .001$ ) influenced by live performance attendance, though the effect size ( $\eta^2 = .049$ ) was minimal. No variables had a significant impact on the participant's preference for the Beethoven VII, second movement excerpt. Gender affected participant preference for

the Witches Sabbath movement from Berlioz's *Symphonic Fantastique* ( $\alpha < .001$ ), though once again the effect size was minimal ( $\eta^2 = .041$ ). Participant preference for Mahler V, fourth movement, was influenced by gender ( $\alpha < .01$ ,  $\eta^2 = .040$ ). Age, gender, musical experience, listening habits, and social variables did not impact participant preference for the Fossils movement of Saint-Saëns' *Carnival of the Animals*. Participant preference for Schoenberg's *Verklärte Nacht* was affected by gender ( $\alpha < .001$ ,  $\eta^2 = .045$ ). In all instances where gender was a significant factor, females showed stronger preference for the musical example than males.

Research question 3 was addressed by determining what relationships, if any, existed between preference, interest, and familiarity. A correlational analysis was used. For the purposes of this study, alpha was set at .05 and moderate ( $r=0.4$  to  $0.7$ ) and strong ( $r=0.7$  to  $1.0$ ) were considered meaningful, even though some correlations were significant at lower levels. The preference for each example was checked against the familiarity and interest for that example.

Significant relationships were found between example preference and familiarity for all but the Mahler (Beethoven V,  $r = .120$ ; Beethoven VII,  $r = .256$ ; Berlioz,  $r = .167$ ; Saint-Saëns,  $r = .132$ ; Schoenberg,  $r = .271$ ). None of these fell in the moderate range of relationships. Moderate and significant correlations were found between each example's preference and interest (Beethoven V,  $r = .601$ ; Beethoven VII,  $r = .682$ ; Berlioz,  $r = .636$ ; Mahler,  $r = .641$ ; Saint-Saëns,  $r = .577$ ; Schoenberg,  $r = .681$ ). Significant relationships were found between each example's familiarity and interest, but none fell in the moderate or strong range (Beethoven V,  $r = .129$ ; Beethoven VII,  $r = .232$ ; Berlioz,  $r = .093$ ; Mahler,  $r = .091$ ; Saint-Saëns,  $r = .120$ ; Schoenberg,  $r = .200$ ).

## Discussion

The results of this study support previous findings (Leblanc, 1981; Leblanc and McCrary, 1983) concerning the relationship between tempo and preference. Though no correlations were used to determine the extent of these relationships, mean preferences show a clear bias toward faster tempi. The two most highly rated pieces were Saint-Saëns' *Carnival of the Animals*, *Fossils* (half note equals 116 bpm), and Beethoven V, fourth movement (half note equals 98 bpm), which were both faster selections. The two slowest selections, Schoenberg's *Verklärte Nacht*, fourth movement (quarter note equals 60 bpm) and Mahler V, fourth movement (quarter note equals 15 bpm), were the two least preferred examples. This trend was not followed by the two examples whose scores fell in the middle. Beethoven VII, second movement is slower (quarter note equals 63 bpm) than Berlioz's *Symphonic Fantastique*, *Witches Sabbath* (quarter note equals 168). This finding may be explained by other variables, such as the differences in complexity, melody, and instrumentation between these two excerpts.

The preference results of the SSMPQ do provide some information on the importance of melody. The more

popular examples, including the Saint-Saëns, Beethoven V, and Beethoven VII selections, all feature prominent and clear melodies. The less favored examples had less obvious melodies, such as the lack of a clear melody in the Schoenberg selection. However, the Mahler and Berlioz examples both had clear melodies that served as the focal point for the example. Possible reasons for their lower preference ratings, in terms of melody, could be due to the position of the melody in the Berlioz example in the brass section, and the observation that the melody in the Mahler example is very slow to develop.

A dearth of research on the impact of mood or modality on preference provides little information on how these variables might be related. The results of the SSMPQ also give very little insight into the impact of mood or modality. The keys of the excerpts were often unclear and a variety of modulations occur that do little to establish a tonal focus. The three more preferred examples (Saint-Saëns, Beethoven V, and Beethoven VII) are in different tonalities (G minor/Bb Major, G Major, and A Minor, respectively), so no clear pattern can be determined.

The importance of rhythm, much like melody, can be seen in the preference results of the SSMPQ. The examples with higher preference ratings (Saint-Saëns, Beethoven V, and Beethoven VII) have clear and simple rhythmic structures, while the less preferred examples (Berlioz, Schoenberg, and Mahler) have less clear or slower rhythmic structures. The use of rhythmic motives, which are found in the three most preferred examples, may also play a large role in catching student attention and making the rhythm more easily perceptible. The Saint-Saëns example features a repeating rhythmic figure consisting of four eighth notes followed by a quarter note (Figure 2). This motif repeats throughout the example. The rhythmic structure of the Mahler example is, on the other hand, very slow to develop and no obvious patterns occur in the selection (Figure 3). These findings match those of previous researchers, who have reported that clear, pattern-based rhythms were preferred (Prince, 1972).



Figure 2: Example of Rhythmic Motif in Saint-Saëns, *Carnival of the Animals*, Fossils



Figure 3: Example of Rhythm in Mahler V, Fourth Movement

An analysis of the preference ratings of the six examples on the SSMPQ shows a clear preference toward harmonically predictable music. The three highest rated examples (Saint-Saëns, Beethoven V, and Beethoven VII) all shared predictable harmonic features, while the three less preferred examples (Berlioz, Schoenberg, and Mahler) had less straightforward harmonic patterns. The harmonic patterns of the Saint-Saëns and Beethoven examples were more conventional and thus more easily comprehended in a single listening than the more complex harmonic movement of the Berlioz, Schoenberg, and Mahler examples. An example of this can be seen when contrasting the Saint-Saëns example with the Berlioz example. In the Saint-Saëns example, the harmony is clearly stated through string pizzicatos and percussion chords. However, the constant movement and scalar passages included in the strings, along with the use of heavy chromaticism, gives an impression of harmonic instability and complexity.

Many examples selected for the SSMPQ included a wide range of dynamic variations. Two of the examples that received the highest ratings (Saint-Saëns and Beethoven V) showed little dynamic activity in comparison with two of the less favored examples (Mahler and Schoenberg). The observation that the more dynamically stable excerpts were more highly preferred than the dynamically varied excerpts may suggest that the use of a variety of dynamics is less important than previously considered (Fung, 1996). However, multiple other variables could have affected preference for the examples. Beethoven V and Saint-Saëns, both dynamically stable and highly rated, were fast, loud, and exciting examples, which contrasted with the dynamically varied but slower Mahler and Schoenberg examples. It is also possible that the brevity of the musical examples emphasized other elements rather than dynamics.

Student preferences, measured by the SSMPQ, favored less complex examples upon initial listening. The Saint-Saëns example, which was the simplest and most straightforward, was the most highly preferred. The most complex example, Schoenberg's *Verklärte Nacht*, was the second-least preferred example. The moderately complex examples, which were Beethoven V, Beethoven VII, Berlioz, and Mahler, fell between these two examples (with the exception of the Mahler example). In this study, the lack of variation in the complexity of the examples allows only limited insight into this relationship, but these results follow the trends discussed by other researchers (Acevedo-Hernandez, 2006).

The responses of the participants in this study do not give clear indications of the importance of instrumentation. The Saint-Saëns example, which featured the xylophone and piano, was the most preferred example, over all of the traditional symphonic examples. The observation that string students preferred these instruments most seems to argue against previous research on instrumental preference, but other factors may have influenced the preference for the Saint-Saëns example (Jin, 1999). This discrepancy may be attributed to the

observation that the Saint-Saëns example differed from the other example on other dimensions and thus may have been of greater interest to the students. Other variables, such as the tempo and simplicity of this example, may explain its high ranking.

On the SSMPQ, the impact of musical experience on preference was assessed through multiple questions in Part Two. Students were asked, "What other instruments do you play?", "Do you take private lessons? If so, how many years have you been taking private lessons?", "Do you play in any other groups or ensembles (orchestras, choirs, rock bands, etc) outside of school?", and "Have you ever attended a live performance of classical music, such as symphony concert or recital?" These questions were included to address experience that the participants may have accrued through their participation in music outside of the classroom. Contrary to Hargreaves, Comber, and Colley's (1995) research, in this study the only significant impact of a musical experience was the influence of the attended live performance variable on preference for Beethoven V, fourth movement. However, only 4.9% of the variance was explained by this relationship. This result is interesting, however, and it suggests that experiencing a live performance may have a positive impact on preference for Western Art music.

The SSMPQ addressed age through a single question that asked students to list their grade. This question gave a rough estimate of both age and the number of years of participation in the school orchestra. The results of the SSMPQ showed that, for these examples, there was no significant impact of age on preference, which contrasted strongly with previous research (Acevedo-Hernandez, 2006; Jin, 1999). Though many studies have found that music preference often varies with age, the examples selected may have appealed to all of the ages included in this study equally. The limited scale and use of the self-report method may have also affected the results of the survey, and students may have rated multiple pieces similarly though they enjoyed each for different reasons.

The results of the SSMPQ suggest that gender has a very strong impact on preference, which supports previous research (Hargreaves, Comber, & Colley, 1995; Jin, 1999). Females ranked the Berlioz, Mahler, and Schoenberg examples significantly higher than males, although the practical significance (2) was quite low (4.1%, 4.0%, and 4.5%, respectively). Higher female preferences for the Mahler and Schoenberg examples, the two slowest, supported Osborn's (1999) claim that females prefer slow Western Art music more than males. However, the higher female preference for the Berlioz example cannot be explained in this way, since the Berlioz example was labeled as fast tempo.

Multiple questions on the SSMPQ addressed the possibility of the social influence on music preference. The questions "Does anyone else in your household play a musical instrument?", "Do your friends listen to classical music outside of school?", and "Does anyone in your household listen to

classical music?" were included to address the possibility of peer and family influence seen in Jin's (1999) study. Though the impact of social influence on preference has been significant in past studies, none of the examples in this study were affected by any of the social variables. A variety of causes could explain these results, including a lack of certainty about friend or family member listening preferences.

Participant preferences for the examples were not influenced by any of the listening habit variables, which were addressed by asking "About how many minutes per day do you spend listening to popular music (rock, country, rap, etc.)?" "About how many minutes per day do you spend listening to classical music?", and "Do you listen to classical music outside of school?" It would seem that students who listen to more Western Art music outside of the classroom would have a greater preference for the style when listening at other times. However, the term "classical," which was used to make the test more participant-friendly and comprehensible, may have included more sub-genres than expected. For example, many students may include popular instrumental soundtracks in the "classical" genre, though this sub-genre was not represented by any of the six examples on the SSMPQ. It is also possible that the style of "classical" music listened to by students outside of the classroom is very different from any of the six examples.

The results of correlational analyses on the data obtained from the SSMPQ revealed that the relationships between preference, interest, and familiarity were significant. Moderate relationships between preference and interest continue to support the idea that interest in Western Art music may be generated by enjoyable selections of music of the style. The weak preference/familiarity and familiarity/interest relationships were statistically significant, though practical significance is negligible, which suggests that familiarity contributed less to the formation of preferences for these examples than expected. However, previous studies continue to assert that the relationship between preference and familiarity is strong and important to the formation of musical tastes (Jin, 1999).

### Implications for Practice

Participant responses to the SSMPQ demonstrate a variety of trends as far as student music preference is concerned. Though many trends found in previous research were not found to be significant in this study, a variety of important implications for the introduction of Western Art music to beginning string students can be identified.

It is clear from the results that students enjoy certain characteristics of Western Art music and that they are adept at detecting these differences, despite limited familiarity with the examples. Students tend to enjoy excerpts with faster tempi that they may perceive to be 'exciting' or 'upbeat'. Though females may have greater preferences for slower tempi, both genders and all ages included in this study preferred faster tempi. Excerpts that are chosen as introductory pieces to the

style of Western Art music could have clear, pronounced, and catchy melodies. Excerpts with clear and constant rhythmic structures, as well as developed rhythmic motifs, are more appealing than examples with unpredictable and unsteady rhythm.

Students tend to enjoy Western Art music that is harmonically predictable and straightforward. Large dynamic variance was not favored by students in this study, though excerpts with some amount of dynamic movement were highly preferred. Mildly complex works may be used to introduce Western Art music and the educator should ensure that the examples are straightforward and they capture attention easily without being overly simple. Teachers are encouraged to select pieces featuring large, symphonic instrumentations. Contrasts in instrumentation can also be appealing to students, especially if the students can identify the instruments involved.

It is important to relate these findings to the characteristics of the popular music that students spend the majority of their time hearing. Most of the characteristics of the preferred examples of Western Art music are also shared by modern popular music. Upbeat, fast, and exciting music is often featured on the radio and on television. Rhythms are constant, predictable, and clear, often with each musical section containing a motif that may be used throughout the song. Harmony is usually very simple, often utilizing a minimal number of chords and focusing instead on clear, catchy, and discernable melodies. Due to the use of compression, a technique that minimizes dynamic contrast in favor of overall volume, popular music usually includes minimal dynamic change.

The complexity of popular music may change drastically between genres, though the music heard most frequently on the radio is simple and catchy. One of the largest differences, and thus a difficult barrier to overcome for educators, is the gap created by the contrasting instrumentations of popular and Western Art music. Popular music seldom includes traditional orchestral instruments, though the music educator may easily be able to find interesting popular works that feature strings, winds, and brass. Therefore, popular music featuring traditional instruments can be used as transitional music when introducing the Western Art style.

The application of this information means many things for teachers. Most importantly, it shows that students' preferences vary greatly from piece to piece across the Western Art style, so it is important for teachers to select music that students will enjoy. Isolating pieces with characteristics that young students prefer could be the first step in preparing to introduce new music to students. These characteristics may also be taken into account when choosing music for rehearsal and performance.

One way teachers could gauge long term changes in preference would be to keep a record of student preferences for the pieces they have played. This would provide information that would be helpful to teachers when selecting music that students will most enjoy playing, which could result in a higher

retention rates.

In this study, few fixed-factor variables had effects on student music preferences for each example. The only significant influences on preference were live performance attendance and gender. The music educator has little control over the impact of gender. Being aware of the gender makeup of the students may be a helpful tool in determining the style and tempo of music chosen if the goal is high preference from the most students.

Live concert attendance, however, is something the educator can easily suggest and possibly even require of his or her students, though there is a possibility that the positive impacts of live concert attendance were due to the increase in familiarity that resulted from the event. However, live concerts may offer levels of excitement and intensity, along with visual stimulation, that audio recordings cannot equal. Using available technology, such as DVD recordings of concerts, could also help improve student openness toward Western Art music, though this has not been explored.

The lack of significant effects of other musical experience variables, age, and listening habit variables suggest that educators may introduce Western Art music to students of varying experience levels, ages, and musical tastes. The lack of any social impact on music preference also provides hope that students in the string classroom, who have already shown willingness to play instruments that are less popular, are more open to new styles of music. Encouraging these students to listen to Western Art music outside of the classroom and with friends could provide another entry point for the beginning string player.

Enhancing familiarity by teaching lessons, using discussions, or involving the students in listening can also be powerful ways to improve the preference for Western Art music. The strong correlations between preference and interest solidify the notion that people will become more interested in music they enjoy. Playing enjoyable Western Art music for students is very likely to increase interest in the style and have a positive influence on students' musical tastes. Combining the characteristics of preferred music from research question 1 with repeated exposure to Western Art music over time could make a significant impact on students' music preferences and openness to new styles.

### Implications for Future Research

The incomplete picture of the function of music preference in the listening process provides many opportunities for future research in the areas of music characteristics, musical exposure, and listener variables. For example, many aspects of musical characteristics have not been explored fully. The areas in which study is most needed are harmony and mood/modality, though other areas are lacking in support as well. Characteristics that are easy to measure, such as tempo and dynamics, have been studied extensively, but more vaguely defined facets such as complexity and harmony are explored



much less often. The definition of harmony is somewhat open to interpretation, since harmony may also be related to complexity or mood/modality. Studies are needed to analyze the impact of harmony and the multiple aspects of harmonic function on student preference.

A distinct lack of research on the effects of mood/modality on student preference is also evident. Preferences for certain modalities may have significant impacts on an individual's preference for a musical example. Students may prefer selections with certain modal characteristics, but it is also possible that modality makes up only a small part of how students determine the mood of a work. Examining the relationship between modality and mood and isolating examples of music with enjoyable moods would provide important information to aid in the educator's music selection process.

Melody has also received very little attention from researchers. To better discuss the importance of melody in music preference formation, studies could be carried out that isolate melodic characteristics. Determining what characteristics students prefer in melodies would be an effective first attempt at understanding preference for melody. Once the importance of melodic elements has been assessed, researchers could isolate musical works that contain these types of melodies and provide information on how to select repertoire with melodies that are enjoyable for students.

The majority of preference studies have been conducted with the preference of each example in mind. The inclusion of a rating system for each variable, such as a Likert-type scale for tempo, complexity, etc., could give more definitive and clear results on how each characteristic contributes to overall preference.

There is a tendency for researchers who focus on style preferences to include a small number of examples to represent the whole of classical or Western Art music. However, students' ideas of classical music are often very broad, occasionally including modern soundtracks or any music whose instrumentation is traditional. Carrying out a study that includes more popular and familiar types of Western Art music, such as movie soundtracks, could help provide insight into how students form preferences for the style.

Familiarity, training, and teacher effects have received considerable attention from researchers. Long-term studies of familiarity, rather than short, intense exposures, should be conducted to see how familiarity with similar music could improve preference for a style.

Studying the effects of live performance could also yield interesting results for researchers. The live performance environment may have an impact on student preferences for Western Art music. The possibility that familiarity is the cause of any preference gains from live performance attendance should be addressed. The study of live performance attendance may also define familiarity's role in the possible benefits of

concert attendance. The effects of audio only, audio and visual, and live performances should be studied in order to understand how preferences may be shaped by different media.

The impact of individual characteristics has also been studied thoroughly by researchers. However, more research on social factors would help to clarify the social position music and how the opinions of others contribute to the individual's music preference. Studying the impacts of society on music preference across many cultures would be an interesting way to compare how culture defines and influences preference.

The results dealing with gender in this study should be further explored. Qualitative research to determine why females tend to have greater preferences for Western Art music and music with slower tempi should be carried out. The unexplained reason for the significant difference between male and female preference for the Berlioz example could also be further explored. This could help educators understand how students listen to music and how the social pressures placed on adolescents can impact music preference.

Longitudinal studies that focus on how preferences change over time with advancing participant age could also yield important results. Determining how students who are exposed to Western Art music early in the classroom develop preferences for the style could have a major influence on the methods teachers use to introduce Western Art music.

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### Mentor Comments

Joshua A. Russell describes the originality and practical implications of Michael Bollero's work as follows:

*Michael recently concluded his research in which he explores the musical preference of secondary (grades 6-12) string students. The purpose of this research was to examine what facets of Western Art music are most appealing to students and what music sparks the most interest. The application of this research would help public school orchestra directors select music that would be liked by young string students, leading to improved motivation and finally greater interest. This is an important study for the field of string music education that could have pragmatic implications for practice.*

*This work was based on a large body of literature on musical preference and interest in music. Michael's research, however, was original in that it was the first of its kind to focus on the population of orchestral string students in K-12 schools. This is an important addition to the literature that could have an impact on future research and practice.*

*Michael's work is worthy of publication because it is original, meaningful, well documented and reported, and could improve musical instruction in string music classrooms. The University of Arkansas should be proud to have such burgeoning research talent in an undergraduate student.*

### Appendix: The String Student Music Preference Questionnaire

Complete each section by circling the appropriate response:

Example 1:

How much do you like this example?

1	2	3	4	5
Dislike very much			Like very much	

Have you heard this piece of music before?

No      Yes

Does this example make you want to listen to more classical music or music like this?

No      Yes

List any aspects or parts of the music that you really liked:

What grade are you in right now? \_\_\_\_\_

Are you:    Male      Female (circle one)

What instrument do you play (circle one)?

Violin    Viola    Cello    Bass

What other instruments do you play?

Do you take private lessons? If so, how many years have you been taking private lessons?

Do you play in any other groups or ensembles (orchestras, choirs, rock bands, etc) outside of school? Please list:

Does anyone else in your household play a musical instrument?

No      Yes      If so, who? \_\_\_\_\_

About how many minutes per day do you spend listening to popular music (rock, country, rap, etc.)?

About how many minutes per day do you spend listening to classical music?

No or Yes

Do your friends listen to classical music outside of school?

No or Yes

Does anyone in your household listen to classical music?

Have you ever attended a live performance of classical music, such as symphony concert or recital?

No or Yes

## INFLUENCE OF PROLINE UPON THE FOLDING AND GEOMETRY OF A MODEL TRANSMEMBRANE PEPTIDE

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**Abstract**

*The orientations, geometries and lipid interactions of designed transmembrane (TM) peptides have attracted significant experimental and theoretical interest. Because the amino acid proline will introduce a known discontinuity into an alpha-helix, it is important to measure the extent of helix kinking caused by a single proline within an isolated TM helical domain. For this purpose, acetyl-GWWLALALAP<sup>10</sup>ALALALWWA-ethanolamide was synthesized, and pairs of deuterated alanines were included by using 60-100% deuterated fluorenylmethoxycarbonyl-L-alanine (fmoc-d4-L-Ala) at selected sequence positions. Solid-state deuterium (<sup>2</sup>H) magnetic resonance spectra from oriented, hydrated samples (1/40, peptide/lipid; using several lipids) reveal signals from many of the alanine backbone C $\alpha$  deuterons as well as the alanine side-chain C $\alpha$  methyl groups, whereas signals from C $\alpha$  deuterons have not been observed for similar peptides without the proline. It is conceivable that altered peptide dynamics may be responsible for the apparent "unmasking" of the backbone resonances in the presence of the proline. Data analysis based upon the method known as Geometric Analysis of Labeled Alanines ("GALA") revealed that the peptide helix is significantly distorted due to the presence of the proline. In order to make available valuable additional data points for evaluating the segmental tilt angles of the two halves of the peptide, it may be advisable to substitute selected leucines with d4-alanine. Together the results suggest that the central proline influences not only the geometry but also the dynamics of the membrane-spanning peptide. The results are important for understanding the functional role of proline in several biological families of membrane proteins, including ion channels and others.*

**Introduction**

Transmembrane proteins are integral components of lipid bilayer membranes and have a variety of functions in biological systems, including acting as channels for ion transport and as receptors for cell signaling. Indeed, about 20-30% of the human genome encodes membrane proteins. In spite of their prevalence, the fundamental molecular interactions between membrane lipids and membrane proteins are poorly understood. For this reason, model systems consisting of designed membrane-spanning peptides are needed to understand the complex properties of biological membranes. The WALP family of peptides is one such model system that is useful for exploring the nature of protein-

lipid interactions (Killian et al., 1996). WALP peptides are transmembrane peptides consisting of the sequence **acetyl-GWW(LA)<sub>n</sub>LWWA-ethanolamide**, in which a variable length core sequence of *n* residues is built using alternating aliphatic leucine (L) and alanine (A) residues. This core generally assumes an alpha-helical configuration (Van der Wel et al., 2002).

The tryptophan (W) residues at either end of the peptide interact with the lipid bilayer's membrane/water interface, producing an anchoring and stabilizing effect on the peptide helix within the membrane. Peptide anchoring probably is accomplished through hydrogen bonding and orientation preferences of tryptophan's aromatic indole rings with the lipid phosphocholine head groups and/or water. Peptide motion is still allowed somewhat, and more motional freedom is provided for amino acid side chains at the C-terminus due to the necessity for reorientation of the C-terminal indole rings to interact properly with the lipid bilayer (van der Wel et al., 2007). Hydrophobic mismatch, namely the difference between the lengths of the hydrophobic regions of membrane lipids and membrane peptides, has been observed in many studies to have consequences for the orientations and motions of both the lipid and peptide components. Nevertheless, research has shown that WALP peptides in general produce a lower than expected response to hydrophobic mismatch. A possible explanation for this result lies in the presence of the two pairs of anchoring tryptophans.

WALP19 is a 19-residue-long member of the WALP peptide family (with "n" = 6; above sequence). Having no helix-breaking residues, WALP19 remains a regular helix (unkinked) within lipid bilayers formed by several membrane lipids, including phosphatidylcholines with 14-, 16- or 18-carbon fatty acyl chains. WALP19 peptides also exhibit very little tilt (~4°) from the bilayer normal (Figure 1A).

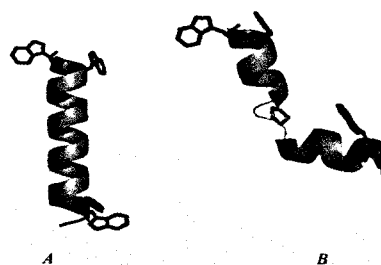


Figure 1: Schematic structures for (A) WALP19; (B) WALP19-Pro (Proline shown in green)

In countless families of biological systems, proline residues buried within the membrane-spanning portion of integral membrane proteins have been found to be highly conserved. For example, membrane ion channels such as the acetylcholine receptor M1, yeast cytochrome b, and the lac carrier of *E. coli* contain proline residues in the central region of the transmembrane portions of the proteins (Cordes et al., 2002). The transport of a substrate through the channel requires a transient, reversible conformational change in portions of the protein. It has been hypothesized that proline's function is to catalyze the cis-trans isomerization of one of proline's peptide bonds within the helix, thus providing the necessary reversible conformational change in the channel protein. In addition, integral non-transport proteins also have been found to contain proline, such as the IKE phage protein and the avian sarcoma virus glycoprotein, and it has been suggested that the proline may serve as a ligand site for protein-cation interactions (Brandl and Deber, 1986). It has also been found that the introduction of a possible kink-inducing proline residue results in a global "loosening" of the entire WALP helix, not just in the vicinity of the proline itself (Demmers et al., 2001). Structures of various proline-containing transmembrane peptides determined by X-ray crystallography have been found to exhibit various kink angles (Cordes et al., 2002) as shown below in Figure 2.

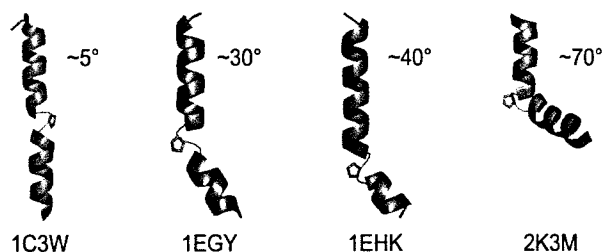


Figure 2. Various kink angles found through analysis of protein crystal structures (Cordes et al., 2002). The labels 1C3W, etc., are code names for structures in the Protein Data Bank (see <http://www.rcsb.org/pdb>).

Despite the highly conserved nature of proline and its potential for a variety of biological roles, the true function of the residue remains to be determined. The presence of proline, with its rigid ring structure, within the transmembrane complex could enhance the response to hydrophobic mismatch. Due in part to its rigid ring structure, and to the removal of a hydrogen bond, proline will tend to interrupt the backbone structure within a standard  $\alpha$ -helix, creating "kinks" (Figure 1B). The 5-membered ring is formed from the aliphatic side chain being joined to both the  $\alpha$ -carbon and the amino group, restricting the torsion angle,  $\Phi$  (Figure 3). In addition, proline is an imino acid and therefore will not hydrogen bond with the ( $i - 4$ ) residue in the peptide sequence (Cordes et al., 2002). The "kinks" that are created by the proline residues are not merely geometric anomalies but are believed to be essential to the voltage-dependent ion transport abilities of several transmembrane protein channels (Sansom and Weinstein, 2000).

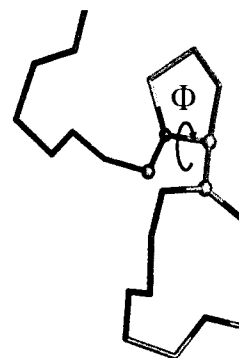


Figure 3. Segment of a peptide backbone, illustrating the conformational restrictions of proline (green).

Within the above framework, an improved method for analyzing the function of proline within membranes is needed. A simplified, model system such as WALP19, within an oriented lipid bilayer, provides a direct method of examining proline's contribution to membrane-spanning segments. Further, solid-state NMR spectroscopy provides a sensitivity that allows for insight into the dynamics as well as the structure of the peptide. To employ this method, the model peptides will need to incorporate deuterium ( $^2\text{H}$ ) labels in specific positions. Combining the WALP-19 model system with solid-state deuterium NMR spectroscopy will enable the determination of whether or not proline has indeed created a kink or influenced the motions within the WALP  $\alpha$ -helix. The results may provide insight concerning the theory that kinking in transmembrane complexes produces greater flexibility for biological function. By altering the amino acids within model membrane-spanning peptide  $\alpha$ -helices, much can be learned about the peptide/lipid interactions within biological membranes.

For the above reasons, I have synthesized WALP19 containing proline as the tenth residue, acetyl-GW~~WL~~AL~~AL~~AP~~AL~~AL~~W~~WA-ethanolamide. Different versions of WALP19-P10 (Figure 1B and Figure 3), were synthesized, each containing two deuterium labeled alanine residues (selected from among the underlined positions). These designed peptides were then analyzed through solid-state deuterium NMR spectroscopy to examine the influence of the proline within the helix.

## Methods

### Preparation of Fmoc-L-Ala-d4

Deuterated L alanine was first derivatized with an N-terminal fluorenylmethoxy-carbonyl (Fmoc) protecting group. Five mmol (445 mg) of deuterated alanine (L-Ala-d<sub>4</sub>, Cambridge Isotope Labs, Inc.) were dissolved in 5 mL water containing 5 mmol (700  $\mu\text{L}$ ) triethylamine (TEA, Mallinckrodt) in a 250-mL round bottom flask. A stir bar was added and the solution was mixed briefly using a magnetic stir plate.

Next, 4.8 mmol (1.62 g) N-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-ONSu) (Novabiochem) was dissolved in 5 mL acetonitrile in a 20-mL

beaker with gentle heating. This solution was added to the pre-dissolved alanine in water/TEA and stirred for 4 hours. TEA was added as necessary during the reaction to maintain the pH between 8.5 and 9.

The mixture was then filtered through a Buchner funnel, with filter paper and the precipitate being discarded. The round bottom flask was rinsed with methanol and the filtrate was returned. The sample was then concentrated on a rotary evaporator until the Fmoc-L-Ala-d4 dried to a thick, yellowish gel.

Sixty mL of HCl (1.5 N) was added to the concentrated filtrate in 10-mL aliquots, while swirling the flask. The flask was then sonicated for 5-7 minutes to further dissolve the pellet. This suspension was then filtered with a Buchner funnel, the HCl poured off, and cold methyl-t-butyl ether (MtBE) was used for rinsing. The cleaned precipitate was then dried on the vacuum line overnight.

A recrystallization step was necessary to obtain a sample of acceptable purity. The Fmoc-Ala-d4 was dissolved in 10-15 mL of ethyl acetate while heating in a water bath. The undissolved material was removed by filtration through a glass frit. The filtrate was added to a crystallization vial and approximately 4 mL hexane (in 1 mL aliquots) was added to the solution. The sample was then put into the freezer for 48 hours and checked periodically for crystals. If no crystals had formed, the volume was reduced by blowing dry nitrogen gas, and the sample was returned to the freezer. Once crystals had formed, they were filtered on a Buchner funnel and dried under vacuum overnight.

#### Solid-Phase Peptide Synthesis

The WALP19-P10 peptides were synthesized by "FastMoc" solid-phase synthesis methods using an Applied Biosystems 433A synthesizer (Table 1). Commercially available fluorenylmethoxy-carbonyl (Fmoc) protected L amino acids (Novabiochem) were weighed out and placed into synthesis cartridges. (However, because of its position at the N-terminal, the acetyl-glycine residue does not possess the Fmoc protective group). For the deuterium labels, one cartridge contained 60% Fmoc-Ala-d4 (40% non-deuterated Fmoc-Ala) and the other 100% Fmoc-Ala-d4 (synthesized above). The amino acid residues were assembled into peptides using the automated synthesizer and an Fmoc-Ala-Wang resin (Novabiochem).

The final peptide was then removed from the resin through an ethanolamine cleavage, which is the final deprotection step for the peptide. The peptide-containing resin was mixed in a 20% ethanolamine mixture (8 mL dichloromethane and 2 mL ethanolamine). The mixture was flushed with nitrogen gas, covered with parafilm, and wrapped in foil. The sample was then placed on a mixer for 48 hours.

The resulting slurry was then filtered through a 10-mL glass filter and rinsed 4 times each with 5 mL each time with dichloromethane (DCM) and trifluoroethanol (TFE). In a 250-

mL round bottom flask, the sample was placed on a rotovapor and allowed to concentrate to a minimal volume. It was then precipitated overnight in deionized H<sub>2</sub>O in the refrigerator. The sample was then centrifuged at 14,000 RPM for 90-120 min. After a pellet had formed, the supernatant was poured off and the pellet was dried under vacuum.

acetyl-GWWL <u>ALA</u> LAPALALWWA-ethanolamide
acetyl-GWWLALAL <u>AP</u> ALALWWA-ethanolamide
acetyl-GWWLALALAPAL <u>ALA</u> LWWA-ethanolamide

Table 1. Synthetic Peptide Sequences with Ala-d4 residues underlined.

Quantitation steps were performed after dissolving the peptide pellet in 4 mL TFE. Then, 10  $\mu$ L of WALP19-P10 in TFE was mixed with 4 mL methanol. An Agilent 8453 model UV-Visible Spectrophotometer set at 280 nm (absorption wavelength for tryptophan) was used to determine the average molar and mass concentrations. A 20  $\mu$ L aliquot of the peptide was diluted with TFE to the concentration of 1 mg/mL prior to analysis by reversed-phase high-performance liquid chromatography (HPLC). The HPLC sample was analyzed to determine the purity of the peptide. Each peptide produced a single peak, illustrating good sample purity, at an elution time of approximately 4.9-5.0 min (Figure 4A).

From each HPLC sample, 50  $\mu$ L was transferred to a second vial and diluted once more with 450  $\mu$ L of TFE (~0.1 mg/mL, resulting solution). Electrospray ionization mass

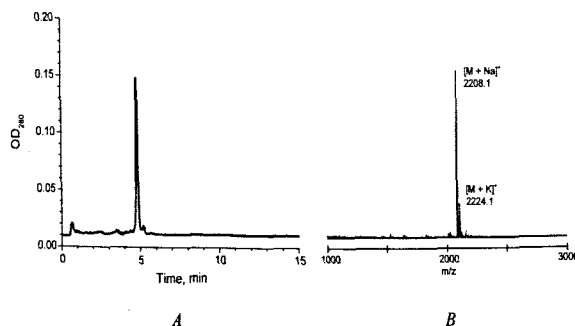


Figure 4. (A) HPLC Analysis of WALP19-P10 and (B) Mass Spectrometry Results for WALP19-P10.

spectrometry was used to verify the peptide molecular mass (Figure 4B).

#### Oriented Sample Preparation

Portions of each synthetic, deuterated peptide were incorporated into hydrated lipid bilayers of dimyristoylphosphatidylcholine (DMPC; 14 C), dilauroylphosphatidylcholine (DLPC; 12 C) or dioleoyl-phosphatidylcholine (DOPC; 18 C). A peptide-lipid mixture at a 1/40 peptide/lipid ratio (95% methanol, 5% water) was evenly applied on 40 glass slides and dried under vacuum (<1.5 Pa) for 48 hours. The slides were

then hydrated 45% (w/w) with  $^2\text{H}$ -depleted water, stacked and sealed in a cuvette. The samples were allowed to orient into a liquid crystalline phase, incubated at 40 °C for at least 48 hours (until the peptide-lipid mixture was no longer cloudy) prior to measurements (Figure 5).

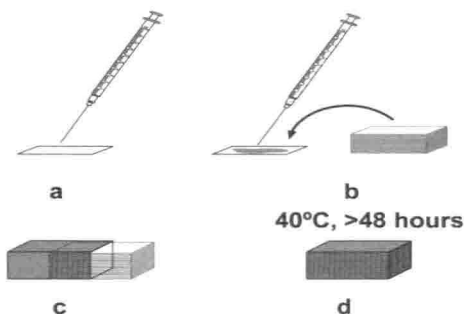


Figure 5. Oriented Sample Preparation (a) Mixture application (b) Hydration and Stacking (c) Slide Placement in Cuvette (d) Incubation at 40 °C.

### Solid-State NMR Spectroscopy

Solid-state NMR technology involves the placement of a sample in a strong magnetic field and sending rapid pulses of radio waves to interact with the molecules. All solid-state nuclear magnetic resonance experiments were conducted using Bruker Avance spectrometers with a proton frequency of 300 MHz. Phosphorus NMR spectroscopy ( $^{31}\text{P}$  nucleus) with broadband  $^1\text{H}$  decoupling was used to confirm that the bilayer lipids were quite well oriented at 323 °K. Spectra were recorded using two sample orientations, with the bilayer normal either parallel ( $\beta = 0^\circ$ ) or perpendicular ( $\beta = 90^\circ$ ) to the applied magnetic field. Spectra of lipid bilayers containing WALP19-P10 were compared to those of pure lipid to determine if the introduction of the peptide was disrupting the bilayer formation.

Deuterium NMR ( $^2\text{H}$ ) experiments were performed at both  $\beta = 0^\circ$  and  $\beta = 90^\circ$  orientations using quadrupolar echo pulse sequence with full phase cycling. Eight hundred thousand free induction decays were obtained for these spectra, compared to only 128 for the  $^{31}\text{P}$  spectra.

### Circular Dichroism Spectra

A circular dichroism (CD) spectrum, obtained using polarized light, indicated that WALP19-P10 maintained an alpha-helical conformation (Figure 6). Of particular importance are the characteristic minima at 208 nm and 222 nm, as well as the peak of maximum intensity near 195 nm.

### GALA Analysis

The  $^2\text{H}$  NMR data of the peptides were analyzed using a program designed in the Koeppel laboratory, GALA (Geometric Analysis of Labeled Alanines) implemented in Microsoft Excel. The quadrupolar splittings ( $\Delta\nu_q$ ) from the spectra were determined; quadrupolar splittings of CD<sub>3</sub> groups in Ala residues are defined as distances between corresponding peaks.

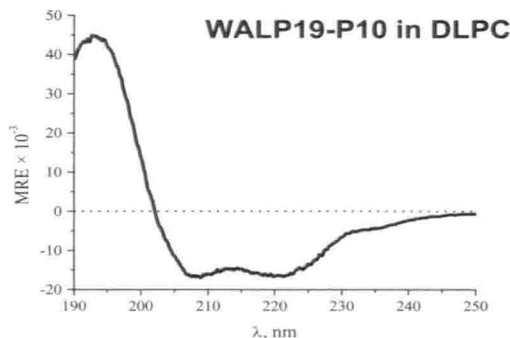


Figure 6. CD analysis is characteristic of an alpha-helix.

The splittings are dependent upon the carbon-deuterium bond orientation, thus providing insights into the structure of the peptide itself. The following expression in Equation 1 shows this relationship:

$$\text{Equation 1: } \Delta\nu_q = \frac{3}{2} S_{\alpha} \frac{e^2 q Q}{h} \left( \frac{1}{2} [3 \cos^2 \theta - 1] \right) \left( \frac{1}{2} [3 \cos^2 \beta - 1] \right)$$

Where:

$\frac{e^2 q Q}{h}$  – static coupling constant (~168 kHz for aliphatic C-D bond)

$S_{\alpha}$  – order parameter (~0.87 for WALP peptides)

$\theta$  – angle between the applied magnetic field and alanine C $\alpha$ -C $\beta$  bond

$\beta$  – angle between membrane normal and magnetic field direction ( $0^\circ$  or  $90^\circ$ )

$\theta$  depends on the orientation of the peptide itself and is expressed by the following relationship:

Equation 2:

Where:

$\tau$  – tilt of the peptide  $\theta = f(\tau, \rho, \epsilon_{ij})$

$\rho$  – rotation of the peptide with respect to the C $\alpha$  of Gly<sup>1</sup>

$\epsilon_{ij}$  – angle between alanine C $\alpha$ -C $\beta$  bond and the peptide axis

The  $\Delta\nu_q$  term is observable and all other variables are constant except  $\theta$ . This is a variable term dependent upon  $\tau$ ,  $\rho$ , and  $\epsilon_{ij}$ . Using these relationships, the GALA program varied the  $\tau$ ,  $\rho$ , and  $\epsilon_{ij}$  values to give the best agreement between the calculated and observed quadrupolar splitting values,  $\Delta\nu_q$  (Van der Wel et al., 2002). A 3-D matrix was created in Excel and using the Grid-Search method, the terms with the lowest root-mean-squared deviation (RMSD) values were determined, indicating the best-fit between the calculated and observed splittings.

Helical wheel plots were then produced that illustrated the RMSD values (or the average difference between the observed and calculated values) as well as the C $\alpha$  positions for each alanine label (in degrees).

### Results

#### Phosphorus ( $^{31}\text{P}$ ) NMR

The phosphorus NMR spectra indicated that the lipids were orienting well into parallel bilayers. At  $\beta=0^\circ$ , this was shown by a tall peak (oriented peak) at approximately 20-23 ppm depending upon the lipid. A much smaller peak at approximately -20 ppm indicated a small fraction of the lipids were not orienting (non-oriented peak), probably representing randomly oriented bilayers. Nevertheless, the smaller peak was seen in both pure and peptide-containing lipid samples, indicating that the peptides were not disrupting the bilayer (Figure 7). All samples produced comparable spectra.

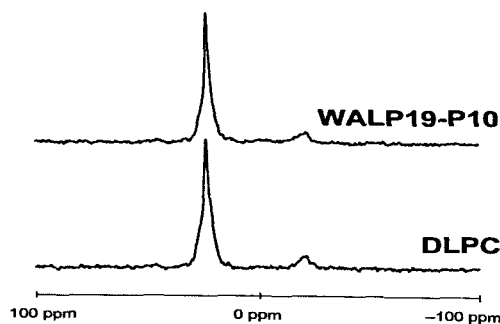


Figure 7. Phosphorus NMR data indicating that lipid bilayer orientation for di-lauroylphosphatidylcholine (DLPC) is maintained in the presence of WALP19-P10, at  $\beta=0^\circ$

### Deuterium ( $^2\text{H}$ ) NMR Spectra

Deuterium NMR results produced two sets of peaks for the  $\text{CD}_3$  groups of the two different labeled alanines in each designed peptide, the taller being representative of the 100% label, and the shorter peak of the 60% label. Figure 8 shows the  $^2\text{H}$  NMR results for samples oriented at  $\beta=90^\circ$  in each of the lipids.

The quadrupolar splitting values were determined as the distances between each symmetrical pair of peaks in each spectrum. These values for the deuterated alanine side chains in



Figure 8.  $^2\text{H}$  NMR results for deuterated alanines in WALP19-P10 in three lipids, shown at  $\beta=90^\circ$ . The lipids are di-lauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMP), and dioleoyl-phosphatidylcholine (DOPC). The numbers at top refer to alanines in the peptide sequence that were deuterated to a level of 100% (large boxes) or 60% (small boxes).

WALP19-P10 are tabulated in Table 2. The large changes in the quadrupolar splittings that are induced by proline-10 indicate that the proline has significant influence upon the helix geometry and orientation.

Ala	DLPC			DMPC			DOPC		
	L10	P10	$ \Delta\Delta\nu_q $	L10	P10	$ \Delta\Delta\nu_q $	L10	P10	$ \Delta\Delta\nu_q $
5	7.2	17.3	10.1	6.6	17.4	10.8	4.4	16.2	11.8
7	3.3	2.6	0.7	7.8	0.0	7.8	11.4	6.0	5.4
9	11.3	18.6	7.3	8.7	17.7	9.0	7.0	13.2	6.2
11	2.6	10.7	8.1	4.6	8.5	3.9	6.8	3.7	3.1
13	14.0	24.3	10.3	12.8	25.2	12.4	11.9	23.2	11.3
15	0.0	15.7	15.7	0.0	16.4	16.4	1.5	13.0	11.5

Table 2. Quadrupolar splittings values ( $\Delta\nu_q$ ) for alanine methyl side chains from  $^2\text{H}$  NMR spectra. The L10 columns refer to WALP19 itself (van der Wel et al., 2002). The  $|\Delta\Delta\nu_q|$  columns show the absolute difference in signal between WALP19 and WALP19-P10.

### $\text{C}\alpha$ Peaks

A surprising result in the  $^2\text{H}$  NMR analysis was that peaks believed to correspond to deuterons attached to the backbone alpha carbons ( $\text{C}\alpha$ ) of the deuterated alanine labels were visible in many of the spectra (Figure 9). This was unexpected as they had never been seen before in the WALP19 peptides lacking the proline residue (van der Wel et al., 2002). Due to the nature of solid-state NMR which utilizes pulses of radio waves, it is likely that the presence of proline is producing some type of change in the nature or time scale of peptide motion. Explanations for this observation are being considered, though it is impossible at this time to ascertain the exact cause for this unique observation. For these reasons, nevertheless, and because the signals were weak, the quadrupolar splittings for the  $\text{C}\alpha$  peaks were not included in the GALA analysis at this stage of the project.

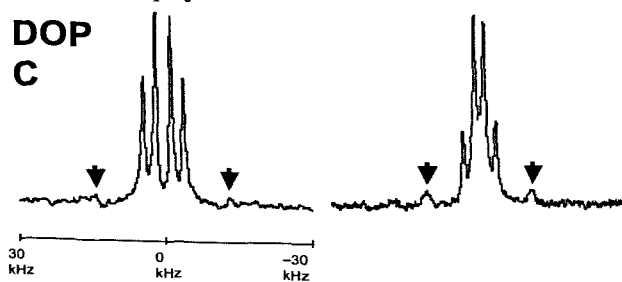


Figure 9.  $^2\text{H}$  NMR spectra illustrating the possible presence of  $\text{C}\alpha$ -D signals from selected WALP19-P10 samples in dioleoylphosphatidylcholine (DOPC).

### GALA Analysis

The GALA analysis was attempted by treating WALP19-P10 as a single, non-distorted  $\alpha$ -helix. It was not surprising to find that doing so produced high root mean squared deviation (RMSD) values. The resulting very poor agreement between observed and calculated quadrupolar splitting values (Figure 10) confirmed a disruption within the  $\alpha$ -helix, illustrating proline's role as a "helix-breaking" residue. It should be noted that the curves in Figure 10 represent the "best" fits to the data. Even so, many of the



data points fall far from the curves in Figure 10, making it impossible to fit a regular alpha helix to the deuterium NMR data. This feature represents the key evidence that proline is distorting the helix. Residue 9, in particular—immediately preceding proline and adjacent to the missing hydrogen bond—fit very poorly and therefore was removed from the data set. Nevertheless, the exclusion of the 9th residue continued to result in unsatisfactorily high RMSD values: 2.5 kHz (DLPC), 3.0 kHz (DMPC), and 3.2 kHz (DOPC). This analysis suggests that proline significantly distorts the position of not only the 9th but also other residues as well.

## Discussion

From a survey using X-ray crystal structures of transmembrane  $\alpha$ -helices in transmembrane proteins, Cordes et al. (2002) found that the presence of proline, which was most often found near the center of the  $\alpha$ -helices, was correlated with kinks (referred to as “hinges”) in the systems. One way in which the authors compared the systems was to align them at the proline residue. When this was done, the C-termini showed good alignment beyond the aligned five-membered proline rings; however, the N-termini exhibited a wide variety of directions and kink angles, ranging from 5° to 70° and demonstrating the increased complexity for this portion of the proteins (Cordes et al., 2002).

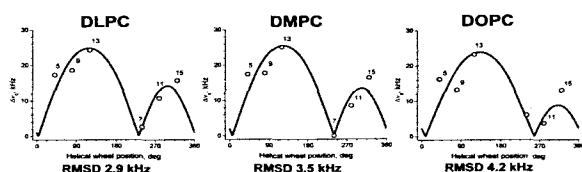


Figure 10. Helical wheel plots for each of three lipids based upon GALA analysis using a canonical undistorted helix. The lipids are dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), and dioleoylphosphatidylcholine (DOPC).

The results from  $^2\text{H}$  NMR analysis for WALP19-P10 agree with the general picture found by the survey of Cordes et al. (2002) and confirms the function of proline as a helix-breaking residue. Indeed it was impossible to perform a complete GALA analysis for the six labeled core alanines when treating the peptide as one single  $\alpha$ -helix. The presence of the proline at the central position 10 disrupts the helix and disrupts the analysis. To proceed further, it will be interesting to determine individually the tilt of each half of the WALP19-P10 peptide, before and after the proline. The individual segmental tilt values will enable us to evaluate the magnitude of the proline-induced “kink” in WALP19-P10. To complete such an analysis, definitively, will require more than three labeled alanines in each segment. For this purpose, I would suggest introducing some single mild mutations, thus changing Leu to d4-Ala at several selected sequence positions, but changing only one leucine at a time. As a control, one already known alanine (data in Figure 8 and Table 2) could be deuterated along with the newly introduced alanine (also deuterated, but with a different

extent of isotope enrichment).

A strategy of introducing additional deuterium-labeled alanines (by leucine replacement) could potentially allow the segmental tilt to be determined for portions of WALP19-P10 before and after the proline. This approach also could allow the kink angle induced by proline to be evaluated in several different lipid bilayer membranes.

In fundamental agreement with the finding of Cordes et al. based upon a survey of 199 proline-containing transmembrane helices from protein crystal structures, proline’s effect on the N-terminus of WALP19-P10 is complex. Likely, the disruption in the hydrogen bonding of the N-terminus is a cause for complications, namely the particular lack of fit for Ala-9 within any helix defined by any of the other alanines to date. The presence of what are believed to be the Ca-deuteron peaks further suggest that there is a change in the dynamics of the protein segment, which could be caused by an increase or decrease in the flexibility of the peptide. Hydrogen bonds are responsible for producing the  $\alpha$ -helical structure of the peptide and removal of particular bonds would be expected to have an impact on the protein’s motion. A complete analysis of dynamics as well as peptide geometry will require further research.

An additional approach to solving this problem (if necessary) would be to alter the model system substantially by mutating all of the leucines of the N-terminal portion of the peptide to alanines. The new system would be created with and without the presence of proline at residue 10 in order to compare the two peptides. Then, each alanine could be labeled – single residues or in pairs – and analyzed in the same manner as before. This approach would eliminate any difficulties that could arise due to leucine-to-alanine mutations and might allow a full characterization of the N-terminus.

In conclusion, my results have shown that the presence of proline in the center of an  $\alpha$ -helix causes a disruption in the geometry of the peptide, a “kink,” while still allowing at least a majority of the helical conformation to be maintained. Peptide dynamics also are influenced by proline. Because the effects upon the two halves of the peptide may be different, the introduction of additional deuterated alanine labels should be considered. Complete characterization of the influence of proline within the WALP19-P10/lipid bilayer model system will require more experiments, yet is likely within reach using available methods.

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#### Mentor Comments:

Professor Roger Koeppe uses the term "groundbreaking" in writing about the importance of Rachel Thomas' undergraduate research and the foundation it provides for additional investigations:

*In her undergraduate research, Rachel Thomas did groundbreaking work to characterize the amino acid proline in a model peptide helix that spans a lipid bilayer membrane. The work is of fundamental importance for understanding proteins that function in biological membranes. In addition to characterizing the influence of proline upon the peptide geometry, Rachel found surprising and novel results: Deuterium labels on the alanine backbone alpha carbons (C-alpha deuterons), that had previously been "silent" in the magnetic resonance spectra recorded for related model peptides, seemed to become "unveiled" by the presence of the proline residue. These unexpected findings suggest that proline may alter not only the geometry but also the time scale and extent of motions within the transmembrane peptide helix. Her results therefore pave the way for a series of follow-up experiments. It is notable also that Rachel proposes future strategies for extending her landmark work. Indeed she continued the project several more weeks in order to complete several of the additional experiments that she proposes in the text.*

## THE BEAUTY OF MATHEMATICS AND THE MATHEMATICS OF BEAUTY: CONTINUED FRACTIONS AND THE GOLDEN RATIO

By Jessica Tush  
Department of Music

Faculty Mentors: Loredana Lanzani and Kim Sexton  
Departments of Music and Architecture

### Abstract

*This project begins with a look at the history of simple continued fractions and how we have arrived where we are today. We then move through a study of simple continued fractions, beginning first with rational numbers and moving to irrational numbers. Continuing further in the pursuit of joining mathematics and art, we define the specific continued fraction that gives rise to the Fibonacci sequence and the Golden Ratio  $\phi$  (phi, pronounced "fai"). These two notions form a direct link to art and the properties that we hope to examine.*

*I have taken an analytic approach to showing that the Golden Ratio has been a constant presence in art history, probably as an indicator of aesthetic appeal. I collected, measured, and analyzed works of art from various periods to investigate the extent to which  $\phi$  is hidden throughout these works. I hope to affirm the hypotheses put forth by others throughout history that yes indeed, those works of art that best exemplify Platonic beauty have instances of the Golden Ratio [Hu] [Li] [Ru] [Ma].*

**Jessica Tush won one of three Undergraduate Research Awards in 2009. The full text of her paper can be found at <http://inquiry.uark.edu/>**

**Because this work was interdisciplinary in content and perspective, feedback from two of her advisors is provided below:**

### Mentor Comments:

Kim Sexton describes the work as superb, writing:

*Ms. Tush's provocative thesis is truly interdisciplinary and based on original investigations not attempted by other scholars. While the candidate is a double-major in math and anthropology, her thesis explores the intersection of math and art history, namely, the art of representing human physiognomy and the influence a mathematical rationalization of beauty (the golden section) might have played in depictions of the human figure over the course of 5000 years (i.e., from ancient Egypt to the present). Art history was a subject in which she had no previous training. Ms. Tush took both the math and art history sections of her research equally seriously. I would like to highlight four qualities of Ms. Tush's work which place it among the best research produced by undergraduates (and by graduate students for that matter):*

- *Ms. Tush's account of the evolution of the golden section in art over 5000 years is utterly original research. No scholar has yet considered the issue over a long duration, including a photograph of Barack Obama and glossy portraits of Hollywood celebrities.*
- *The double-major's agile handling of sophisticated theoretical paradigms – mathematical proofs and proportional ideality in art – structures the analyses throughout her thesis. Here, theory is no mere introductory distraction or flourish.*
- *In the formal analysis of a wide-range of portraiture – still the key interpretative tool of art historians – this thesis shows the candidate to be not only competent, but progressive and original.*
- *Finally, what truly sets this thesis apart is Ms. Williams' avoidance of the pitfalls of essentialist reasoning that ensnares many undergraduate researchers. She works deftly with a sliding spectrum of traits rather than giving undue credence to artificially constructed norms of beauty. Ms. Tush's submission is a model of undergraduate research on a topic of fundamental importance – the relationship between science and art – both for the respective disciplines and for society at large.*

And Honors thesis advisor Loredana Lanzani says:

*Jessica's interdisciplinary thesis is a fascinating examination of "The Beauty of Mathematics and the Mathematics of Beauty." It consists of two interconnected parts. In the first part, she has explored the topic of continued fractions, which departs from and significantly expands the course material she covered in my class (Math 2103); as an application of continued fractions, she has then studied the geometric and analytical properties of a special number, the golden ratio, (also known as the divine proportion) which since Classic Greece has been considered the golden paragon in the assessment of beauty. In the second part of her thesis, mentored by Dr. Kim Sexton (School of Architecture) Jessica has been "looking for the golden ratio" throughout a large number of artworks and iconic pop-culture images that most scholars acknowledge to be good representatives of the social perception of beauty throughout different historical periods. She has then compiled various statistics with the goal to quantify the extent to which the social perception of beauty is indeed affected (consciously or subconsciously) by the presence, in some form or other, of the golden ratio.*

*Jessica was by all means the primary investigator throughout all the many aspects of this project. She selected the topics herself, and she developed all the sophisticated mathematical skills that were needed in order to master the subject of continued fractions. Under Dr. Sexton's guidance, Jessica (who is not an arts major, nor an arts minor) selected over 30 artworks and images, found the most suitable software, and learned how to use it to analyze the selected art works and images: based on these data she developed her own set of comparison criteria to evaluate the various artworks and images.*

*The interdisciplinary nature of this project makes this work quite unique and appealing to a wide array of researchers: the mathematician is excited to learn about "real-life" applications of the quite abstract but beautiful tool of continued fractions. The art historian appreciates how a generally acknowledged concept (namely, the idea that the golden ratio has some kind of an effect on the perception and the representation of beauty) is in fact precisely quantifiable and rationally explainable by means of rigorous mathematical analysis. The novelty of this work is the systematic (and highly successful) effort to present a new perspective by bringing the two subjects together in a very in-depth analysis both of the pertinent mathematical techniques and of the artworks.*

*Jessica plans to become a high school teacher. This interdisciplinary project has great potential to be developed into a successful tool for interdisciplinary teaching at the high school level. Throughout the engaging use of computer technology, high school students can be brought to learn and master sophisticated mathematical theorems and at the same time bring "life" into these techniques by using them to investigate their own favorite images and artworks. The potential rewards that this approach can bring to the high school classroom cannot be understated, for instance: a new appreciation for, and a deeper understanding of, an often intimidating subject such as Mathematics. Jessica also has all the personality traits that make a great teacher – talent, passion, great communication skills, enthusiasm for her subject matter, but also patience, the ability to listen, and a sense of humor. I know she will be able to turn this project into a highly effective and at the same time very enjoyable pedagogical tool.*

# MARKET VOLATILITY ASYMMETRIES: THE EFFECTS OF STOCK MARKET RETURNS ON REALIZED AND IMPLIED VOLATILITIES

By Matthew M. Chesnut

Department of Finance

Faculty Mentor: Alexey Malakhov

Department of Finance

## Abstract

*Volatility is an integral and inescapable variable of financial engineering, modeling, and finance theory itself. Classical financial economics proxies volatility for risk itself, as it becomes difficult to predict future price realizations of a given asset when that asset exhibits significant price volatility over a given time. However, the nature of volatility as it is explained by classical financial economics has been extensively questioned in the previous three decades, since it is characterized as a function of uncertainty and aggregate market psychology—that is, as a function of fear, greed, exuberance, and other fundamental human instincts and emotions.*

*While previous research has primarily focused on the asymmetries between stock market returns and realized volatility, this paper examines the extent to which implied volatility is asymmetrical with regards to the nature (positive or negative) of stock market returns in simultaneous periods. Analyses indicated that negative stock market returns create uniquely positive innovations to implied volatility not created during periods of positive stock market return. Additionally, this paper attempts to reconcile the asymmetry in implied volatility back into a cogent behavioral theory. Finally, the analyses described here explore how this asymmetry causes systemic error in predicting innovations to implied volatility and suggests a simple systemic error adjusted VXO model can be utilized with great efficacy to predict future innovations to realized volatility.*

## 1. Introduction

Volatility is an integral and inescapable variable of financial engineering, modeling, and finance theory itself. Indeed, it is a formative building block of option pricing models and sundry portfolio allocation models. Classical financial economics proxies volatility for risk itself—that is, an asset that demonstrates large volatilities of price realizations over a given time series makes predicting future price realizations with certainty very difficult. Classical efficient market paradigm, which posits markets as acting freely and appropriately to all relevant information pertaining to an asset or security's price function, explains volatility as a perfect reflection of market uncertainty. The risk of a given

security is captured within volatility in that the security's price volatility reflects the aggregate uncertainty pertaining to that security's future prospects (Kurz and Motolese, 1999). The nature of volatility as it is characterized by classical financial economics has been questioned extensively in the previous three decades. Research suggests that the various price changes that the U.S. equity markets have experienced since the 1920s cannot be explained merely by the potential variability and uncertainty surrounding the discounted cash flows of future dividend payments (Shiller, 1981). Rather, realized volatility is systematically greater than could be explained by the efficient market hypothesis. Now, many financial economists have posited volatility exists not only as a function of uncertainty, but also as a function of aggregate market psychology—that is, as a function of fear, of greed and exuberance, and other fundamental human instincts and emotions. Perhaps most pronounced among these base emotions and instincts is the human inclination towards loss aversion (Kahneman and Tversky, 1978) and its relationship to volatility innovations (Dennis, Mayhew, and Stivers, 2006). Prospect theory<sup>1</sup> posits individuals as engaging in asymmetric loss aversion. All things held constant, economic losses affect individuals emotionally and psychologically to a greater degree than gains. Individuals thus overcompensate for small probabilities of negative occurrences and assign larger weights to them in their decision criteria than they logically should<sup>2</sup>. Research also indicates that loss-aversion is not static; rather, an investor's loss-aversion function changes during periods of stock return shocks. Thus, the dynamic nature of an investor's loss aversion function can cause volatility feedback loops (Barberis, Huang, and Santos, 2001).

Volatility, however, has not been relegated exclusively to the confines of academia. In 1993 the Chicago Board Option Exchange (CBOE) introduced a volatility index that identified the volatility implied by a perfectly at-the-money option of the S&P 100 for the next 30 calendar days. This would later be named the VXO, as a new volatility index, named the VIX, would be introduced in 2003. In the next year, on March 26, 2004, the CBOE launched a futures exchange where investors and speculators could trade futures on volatility itself, making

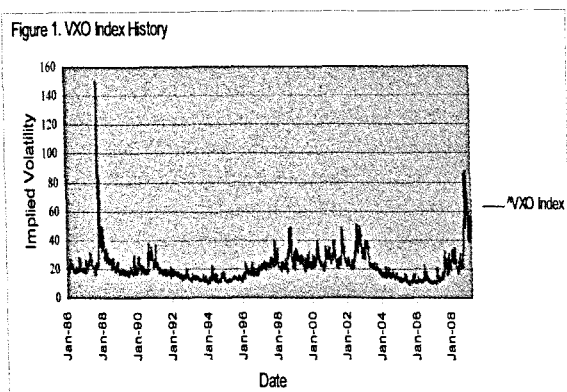
<sup>1</sup> Prospect theory is an explication of how individuals make decisions under risk. It is descriptive in nature, as opposed to optimal (like expected utility theory). The primary difference between the two theories is that expected utility theory posits utility curves as exclusively convex and static. On the other hand, prospect theory posits that, from a given reference point, the utility function of gains is concave, whereas the utility function of losses is convex. Additionally, prospect theory posits utility curves as dynamic—that is, they shift based on the starting point of the individual making the decision (i.e., How wealthy am I? What do I have to lose?) and how the decision and risk is framed in the mind of decision maker.

volatility an asset class in and of itself.

In the seven months since October 2008, the 30-day implied volatility of the S&P 100 (see Figure 1) had reached levels never before seen since the index's creation. Indeed, the VXO has breached levels not observed since Black Monday<sup>3</sup>.

Given these levels, discussions of volatility with regards

Figure 1. VXO Index History



to both its nature and its relationships to other market functions are both important and timely. While realized volatility has been the topic of numerous academic inquiries, this paper further explores the functioning of realized and implied volatility in financial markets, its relation with other market variables, and the accuracy of implied volatility indexes in predicting future realized volatility. Specifically, this paper addresses the following:

- Qualification and quantification of the asymmetries in volatilities that are realized historically and predicted for the future based on the nature of the market returns (positive or negative) that exist contemporaneous to them.
- Investigation of whether the VXO systematically overestimates future volatility, and whether any bias is determined by the nature of contemporaneous market returns.
- Isolation of systematic bias as from the VXO to determine if a superior model for predicting future volatility can be created.

The creation of a unique model for predicting future volatility is of paramount importance in finance, since creating models with greater explanatory power than the market is exceedingly difficult. The ability to identify volatility mispricings, in conjunction with the accurate estimation of future volatility in financial markets, would allow for the development of new, potentially lucrative volatility trading strategies.

## 2. Hypothesis Development

In order to develop research hypotheses related to the issues of volatility described above, it is necessary to examine more closely previous academic research that has postulated asymmetric correlations of volatility with different types of stock market returns. There have been two dominant, though not mutually exclusive, explanations of this presumed phenomenon: volatility feedback effect and the leverage hypothesis. Proponents of the volatility feedback effect explanation rely on the Capital Asset Pricing Model in Modern Portfolio Theory. This model is used to quantify the rate of return a specific asset should have in order to justify its existence in a well-diversified portfolio. All things being equal, an increase in the market risk premium (i.e., how much additional return should be generated by the market in order to justify holding risky assets relative to risk-free assets) requires an increase in firm specific expected return in order to justify an individual security's possession in a portfolio.

Research indicates that the market risk premium is positively correlated with market volatility (Kim, Morley, and Nelson, 2008). Therefore increases in market volatility, and thus the equity risk premium, require an increase in firm specific expected return in order to justify an individual security's possession. Volatility feedback posits that as a firm's and market's volatility increases over a period of time, its value should decrease if its expected future cash flows are held constant. Therefore, increases in volatility typically cause stock price depreciation, which in turn raises volatility even further, creating a volatility feedback loop (Bae, Kim, and Nelson, 2007). In contrast, the leverage hypothesis asserts that asymmetric volatility can be explained by the inherently increasing leverage of a firm (debt-to-market capitalization) as its share price decreases. The volatility of a security's price increases as the firm's leverage also increases. However, asymmetric volatility persists even if the firm is financially unlevered; that is, if the firm has no debt on its balance sheet (Daouk, Hazem, and Ng, 2004).

Few studies have extended the potential asymmetry of returns to innovations in implied volatility. Determining if asymmetry exists in implied volatility innovations relative to stock market returns would allow for a better understanding of the degree to which the aggregate market's loss aversion function changes with stock market returns (both with regard to nature and magnitude). Such analyses would allow for greater clarity regarding both future volatility innovations and the extent to which implied volatility assessments might be systematically biased. The study is grouped into three hypothesis groups. The first, containing four parts, explores whether asymmetry exists between implied volatility, realized volatility, and previous 90-day returns. The existence of asymmetry will be tested where:

<sup>2</sup> Refers to fair actuarial estimates of statistical probabilities of various gains and losses

<sup>3</sup> Black Monday refers to Monday, October 19, 1987. On said date, the S&P 100 fell 21.16% at its closing from the previous Friday's closing.

$H_{0(a)}$ : The relationship between previously realized 30-day volatility is consistent and symmetrical between previous 90-day returns of similar absolute varieties, but of different natures (positive or negative).

$H_{1(a)}$ : The relationship between previously realized 30-day volatility is inconsistent and asymmetrical between previous 90-day returns of similar absolute varieties, but of different natures (positive or negative).

$H_{0(b)}$ : The relationship between implied volatility and previous 90-day returns of similar absolute varieties, but of different natures (positive or negative) is consistent and symmetrical.

$H_{1(b)}$ : The relationship between implied volatility and previous 90-day returns of similar absolute varieties, but of different natures (positive or negative) is inconsistent and asymmetrical.

The second and third hypothesis groups question whether the VXO overestimates future realized volatility, if the overestimation is symmetrical with regard to the nature and magnitude of returns of previous 90-day periods, and if any systematic overestimation can be factored out to produce a superior model for estimating future volatility, where:

$H_{0(c)}$ : The VXO does not have a systematic error residual.

$H_2$ : The VXO has a systematic error residual that is substantially affected by the nature and magnitude of the return of the previous 90-day period. The error that occurs during periods where previous 90-day returns were positive are statistically different than the error that occurs during periods where previous 90-day returns were negative.

$H_{0(d)}$ : Adjusting for systemic errors of each variety (systematic error of returns of a positive nature for time periods where previous 90-day return was positive, systematic error of returns of a negative nature for time periods where previous 90-day return was negative) as it is perceived does not render a superior model for predicting future stock market volatility.

$H_3$ : Adjusting for systematic errors of each variety (systematic error of returns of a positive nature for time periods where previous 90-day return was positive, systematic error of returns of a negative nature for time periods where previous 90-day return was negative) as it is perceived renders a superior model for predicting future stock market volatility than other simple models.

$H_3$  is of particular importance. Predicting future volatility significantly better than the market's prediction is very difficult but could also be exceedingly profitable. The models constructed hereafter, and their concomitant analysis, could be used to identify and take advantage of future volatility mispricings in financial markets.

### 3. Methodology

This paper uses reverse-engineered VXO values dating

back to January, 1986, and the 30-day realized volatility of the S&P 100<sup>4</sup> dating back to 1982 in order to explore three specific topics. First, the analysis tests the extent of correlation between stock market returns and volatility of both a realized and implied nature. Within the scope of this topic, the extent of correlation between 90-day positive returns and 30-day realized and implied volatility is analyzed against correlations between 90-day negative returns and 30-day realized and implied volatility to determine if volatilities are symmetrical between returns of a similar absolute degree, but of a different nature. This is accomplished via a multivariable regression where previous 30-day realized volatility, 90-day S&P 100 absolute returns, and the sign of the return of previous 90-day S&P 100 returns are utilized to explain the variability of VXO innovations. This regression utilizes a format where  $Vol_t$  is the previous 30-day realized volatility,  $c_{Vol}$  is the coefficient of realized volatility,  $AbsRet_t$  is the absolute return of the S&P 100 over the previous ninety days,  $c_{Abs}$  is the coefficient of the absolute return,  $Return\ Sign_t$  is the nature of the return (positive or negative) of the S&P 100 over the previous ninety days,  $c_{Return\ Sign}$  is the coefficient of the return nature and  $\varepsilon$  is the error term.

$$VXO = c_{Vol} Vol_t + c_{Abs} AbsRet_t + c_{Return\ Sign} Return\ Sign_t + \varepsilon$$

Both the absolute return and the sign of the return over the previous 90 days are included to account for innovations to implied volatility that might be derived from the magnitude of the return alone. Therefore, the  $Return\ Sign_t$  coefficient is used to demonstrate the degree of asymmetry derived by the nature of the return itself, positive or negative.

Second, this paper utilizes a Mann-Whitney U test to determine if the error residuals of VXO in predicting future volatilities during periods of negative stock market returns come from the same distribution as future volatilities during periods of positive stock market returns. A rejection of the null hypothesis indicates that the VXO is systematically biased in its estimation of future volatility based on the nature of the previous 90-day returns.

Last, five models (the VXO, an error-adjusted VXO, realized 30-day volatility, an error-adjusted 30-day volatility, and an equally weighted index composed of VXO and realized 30-day volatility) are analyzed to determine their respective efficacy in predicting future volatility. The error-adjusted VXO,  $VXO_{adj}$ , is constructed by adjusting each VXO reading at time, by the average error term experienced up to that time period by error type and return nature. Therefore,  $\varepsilon_{(+)}$  is the mean error term up to that period for all VXO readings that are contemporaneous with positive previous 90-day returns,  $90\text{-return}_{(+)}$ ,  $\varepsilon_{(-)}$  is the mean error term up to that period for all VXO readings that are contemporaneous with negative previous 90-day returns,  $90\text{-return}_{(-)}$ .

$$\text{If } 90\text{-return}_{(+)} : VXO_{adj} = VXO - \varepsilon_{(+)}$$

<sup>4</sup> Realized Volatility is determined by calculating the standard deviation of daily lognormal returns over the previous 30 calendar days, or 21 trading days, and then annualizing the return. Thus,  $\text{Realized Volatility} = \sigma\{\ln r_t : \ln r_{t-30}\} \cdot \sqrt{252}$ .

If  $90\text{-return}_{(-)} : \text{VXO}_{\text{adj}} = \text{VXO} - \varepsilon_{(-)}$

The five models are evaluated by a number of criteria, including their explanatory power (represented by  $R^2$ ), their respective mean squared error, the kurtosis of each model, the mean error of each model in order to determine systematic bias, and the range of each model's errors, among others.

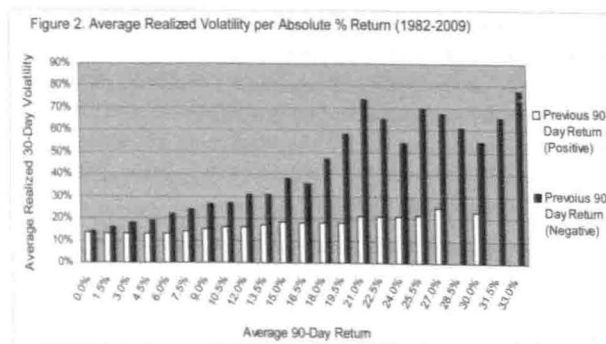
## 4. Results

### 4.1. Asymmetric correlation between realized volatilities and returns of different natures (positive or negative) of previous 90-day periods.

The degree of correlation between returns of a positive variety and realized volatility, and returns of a negative variety and realized volatility, is significantly asymmetric. However, returns and correlation are not inversely related, as popularly pronounced by stock market pundits. Indeed, on average, volatility increases as absolute stock market returns go up (in either a positive or negative manner). Additionally, substantial asymmetry exists between volatility and the type of return (positive or negative) experienced over previous 90-day periods (see Figure 2). Volatility is mildly positively correlated to the degree of positive stock market returns over previous 90-day periods ( $r = 0.17899$ ). Reciprocally, volatility is much more strongly negatively correlated to the degree of negative stock market returns over previous 90-day periods ( $r = -0.71553$ ).

### 4.2. Asymmetry between implied volatility innovations and periods with previous 90-day returns of different natures (positive or negative).

Figure 2. Average Realized Volatility per Absolute % Return (1982-2009)



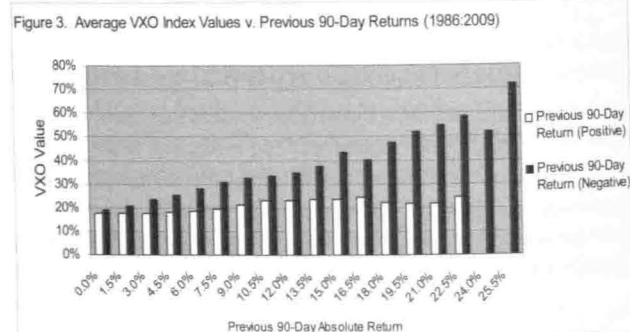
Realized volatilities are highly correlated with implied volatilities of contemporaneous periods ( $r = 0.8792$ ). Because of this, a multivariate regression is used to determine innovations of implied volatility, represented by the VXO as the dependent variable. In this model realized volatility, absolute previous 90-day return, and the sign of previous 90-day return are independent variables. The regression shows that 79.8% of the variability of implied volatility can be explained by changes to previous 30-day volatility, the absolute value of previous 90-day volatility, and the sign (positive or negative) of previous 90-day returns (see Table 1 below). The coefficient of the sign of previous 90-day returns in the regression is -0.0163. Thus,

Table 1. Regression Statistics--Implied Volatility Innovations: 1986-2009

	VXO Index
Intercept	0.0921***
$C_{\text{Vol}}$ (Realized Volatility)	0.6809***
$C_{\text{Abs}}$ (Absolute Previous 90-Day Return)	0.3637***
$C_{\text{ReturnSign}}$ (Sign of Previous of 90-Day Return)	-0.0163***
Adjusted $R^2$	0.79820
N	5838

Significance levels of 10%, 5%, 1% are denoted by \*, \*\*, \*\*\* respectively.

Figure 3. Average VXO Index Values v. Previous 90-Day Returns (1986-2009)



returns of a negative variety in previous 90-day returns causes an increase in implied volatility of 1.63 percentage points, all things held equal, compared to returns of a positive variety in previous 90-day returns. The asymmetry of implied volatility innovations to periods of varying natures and magnitudes of previous 90-day returns is also visually represented in Figure 3 below, which plots previous 90-day returns against average VXO readings for that return interval.

### 4.3. The error term of the VXO is systematically biased, and is asymmetric with regards to the nature of the previous 90-day return.

The descriptive statistics (see Table 2) of the VXO error terms shows that the VXO consistently overestimates future volatility. On average, it predicts volatility being 4.65% higher than is realized over the next thirty days. The systematic overestimation of future volatility is further determined by an analysis of the kurtosis of the error terms. Indeed, the kurtosis of the sample indicates that most of the variance of the error term is due to extreme observations in the data set. The results of the Mann-Whitney U Test (see Table 2) indicate that the error terms resulting from VXO readings contemporaneous with previous 90-day returns of a negative nature have a statistically different distribution than error terms resulting from VXO readings contemporaneous with previous 90-day returns of a positive nature. The differences in distribution of VXO error terms derived from periods exhibiting positive and negative stock market returns is further augmented by analysis of the descriptive statistics of the VXO error terms disaggregated by previous 90-day return nature (see Table 3). These findings are consistent with the findings of 4.2



above. Since previous 90-day returns of a negative nature are associated with unique positive implied volatility innovations that are not shared by previous 90-day returns of a positive nature, it could be expected that any systematic bias of implied volatility could be further exacerbated during periods of previous 90-day negative returns.

#### 4.4. Error adjusted VXO model predicts future volatility innovations with greatest accuracy.

The predictive power of five different models is of value in determining whether effective future predictions of implied

Table 2. Descriptive Statistics and Mann-Whitney U Test: VXO Error

Panel A. Descriptive Statistics of VXO Error Terms	All	Positive Returns	Negative Returns
Mean	0.04646	0.04356	0.05137
Median	0.05030	0.04831	0.05446
Standard Deviation	0.08125	0.06619	0.10161
Kurtosis	46.01072	76.24246	26.86632
Range	1.72564	1.10920	1.71910
Minimum	-0.82971	-0.82971	-0.82317
Maximum	0.89593	0.27950	0.89593
Count	5825	3662	2163

Panel B. Mann-Whitney U Test	Return Variety	N	Mean Rank	Sum of Ranks
Error	Positive	3,662	1877.96	10,997,386
	Negative	2,163	1025.03	5,970,811

Panel C. Mann-Whitney Test Statistics	
Mann-Whitney U	3,690,047
Z	-5.32115
P (Two Tailed)	0.0001343

volatility can be made, as predicting future volatility is of great importance in financial modeling. The systematic error adjusted VXO model is superior to the four competing models over the time period analyzed, as it has better explanatory power and less squared error in predicting future volatility than the other four models utilized (see Table 3). Additionally, the error adjusted VXO model has the greatest kurtosis of all models analyzed, implying that a greater amount of the variance of the error can be attributed to more extreme observations compared to the other models utilized. The error adjusted VXO has the smallest range of errors, which means that the scope of its accuracy is greatest compared to the other models. Finally, all calculations of the error adjusted VXO model were performed out-of-sample<sup>5</sup>. This fact grants further legitimacy to the use of this model as a mechanism for predicting future volatility.

As explained previously, the VXO systematically overestimates future volatility. So does the equally weighted index composed of the VXO and previous 30-day volatilities, but by a lesser degree. Reciprocally, no systematic error appears to occur when utilizing previous 30-day volatilities to predict future volatility. However, this model has less explanatory power than either the stand-alone VXO model or the weighted model of the VXO and previous 30-day realized volatilities.

## 5. Interpretations and Conclusions

This paper analyzes the asymmetries of volatilities (both realized and implied) that occur simultaneously with returns

Table 3. Descriptive Statistics of Error Residual--Future Volatility Models

	VXO	Previous 30-Day Realized Volatility	Weighted (VXO & Previous 30 Day Previous Volatility)	Error Adjusted Previous 30-Day Realized Volatility	Error Adjusted VXO
R <sup>2</sup>	0.4526	0.3762	0.4376	0.3816	0.5777
Mean Squared Error	0.0088	0.0086	0.0075	0.0086	0.0049
Mean (Error)	0.0465	-0.0007	0.0229	-0.0007	-0.0045
Median (Error)	0.0503	0.0014	0.0285	0.0013	-0.0022
Standard Deviation (Error)	0.0812	0.0927	0.0635	0.0928	0.0701
Kurtosis (Error)	46.0107	31.8493	39.4415	31.1652	61.2038
Range (Error)	1.7256	1.6109	1.5839	1.6169	1.5549
Minimum (Error)	-0.8297	-0.8613	-0.8455	-0.8407	-0.8834
Maximum (Error)	0.8959	0.7496	0.7384	0.7762	0.6715
Sum (Error)	270.6454	-4.2912	133.1771	-3.7756	-26.0193
Count	5825	5825	5825	5825	5825

of different natures. Based on these analyses, it appears that innovations to volatility are asymmetrically related to the nature of the return existing contemporaneous with it. With respect to implied and realized volatilities, negative returns create excess positive innovations relative to positive stock market returns. Thus, realized volatility increases during periods of negative market return. In this study, the market's aggregate expectation of future volatility is seen to increase in excess of what should be expected from contemporaneous realized volatilities during periods of negative market returns, as well. Further, the error terms of volatility predictions via the VXO are statistically different between volatility predictions made during periods of positive stock market returns and periods of negative stock market returns. Specifically, though implied volatility estimates are consistently positively biased, implied volatility bias during periods of negative stock market returns is substantially larger than implied volatility bias during periods of positive stock market returns.

The analyses of volatility in this paper were performed to clarify whether utility curves in capital markets follow those hypothesized by classical expected utility theory, or if they might instead follow an asymmetric and dynamic model proposed by prospect theory and behavioral finance. The results presented here indicate that systemic overestimation of future volatility occurs during periods of positive and negative stock market return. A systematic overestimation of volatility, in and of itself, would seem to be consistent with a typical concave utility function. Due to the concavity of the function, a given dollar loss affects an individual's utility greater than a corresponding dollar gain. Since volatility is asymmetrically correlated to negative returns, an overpayment<sup>6</sup> for protection from the impact of volatility on an investor's portfolio should make up for the asymmetry of utility change differentials between equal gains and losses and would be consistent with classical expected utility theory. However, the results show that innovations to implied volatility are not static, but dynamic.

<sup>5</sup> The adjusted value for the VXO was calculated by adjusting for the error as the error was perceived in the time series. Thus the amount of change in the model changes as the average error perceived changes, as opposed to a static change imposed on all data determined by the average error over all of the data set.

This is inconsistent with classical expected utility theory, since utility functions should be static. Thus, the degree to which one overpays for the right to speculate towards or protect his portfolio from future volatility per any degree of previously realized volatility should be similar, regardless of the nature of returns that exist contemporaneously.

This asymmetry in innovations to implied volatility, however, is consistent with prospect theory. Since investors will pay more for protection from future volatility during periods of previous negative stock market returns per any given unit of historical volatility, investors' utility functions should change dynamically depending on numerous circumstances affecting myriad unknown personal considerations (health, wealth, happiness, etc.). Indeed, this asymmetry means investors gain more value for protection (or speculation) from volatility during periods of negative stock market return than they gain during periods of positive stock market return.

Finally, the analyses reported here suggest that utilizing the VXO to predict future volatilities, after adjusting for systemic error, provides a much more accurate assessment of future realized volatilities compared to the other five naïve models utilized. Though simplistic, the model shows that systematic overestimations can be isolated from the VXO to render a more accurate assessment of future volatility innovations than other naïve methods utilized. This finding is of considerable importance—both in academics and in the participation of financial markets. The ability to build a model with better explanatory power than the market is very challenging, given the sheer aggregate energy spent attempting to predict market variables. The ability to predict and identify volatility mispricings in capital markets would allow for the development of unique and lucrative trading methods, in concomitance to a better understanding of how volatility is uniquely innovated during different periods of stock market returns.

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## Mentor Comments:

*Research mentor Alexey Malakhov describes the independent work of Matthew Chesnut in pursuing a challenging research topic and generating data that has immediate applications to understanding prediction of market risk. He notes: Matt's thesis addresses the asymmetric nature of investor's perception of risk as quantified by implied volatility. His research rigorously documents the difference in investors' risk perception during good and bad times as measured by previous 90-day market return. This phenomenon can hardly be explained by the classical finance theory, which is the core of our undergraduate curriculum at the Walton College, but it is consistent with psychological and behavioral explanations suggested by Matt. Undoubtedly, the most innovative part of Matt's thesis is his approach that quantifies behavioral biases that are present in the implied volatility estimates of future risk. Furthermore, Matt convincingly proposes a model of predicting future risk that accounts for the different nature of behavioral bias in good and bad times. This is a really innovative and exciting contribution to the current academic finance literature.*

*It is important to emphasize that although Matt worked under my supervision, he came up with all the research ideas in his thesis, as well as with the ways of implementing them. My involvement was limited to providing feedback on his numerous research ideas, and helping him to concentrate on the more promising areas, as well as exposing him to the existing literature and methodology that was not covered in our Finance curriculum. In the course of his research, Matt pushed the envelope of existing academic knowledge by exploring previously unexplained financial phenomena, and trying to come up with rigorous interpretations of the results. As usual in scientific inquiry, not all of Matt's conjectures bore*

<sup>6</sup> Implied volatility is also a proxy for the expensiveness of an option. At any given strike, as the premium to utilize the option increases in price, the implied volatility must also necessarily increase as well. Therefore, the implied volatility of a specific option is the volatility that is required to be experienced by the underlying asset in order to match the price of the strike.

*fruit, and a good deal of projects turned out to be “dry holes”. I personally consider that a testament to the breadth and scope of Matt’s research, as the final version of his thesis represents only a fraction of overall research that he has conducted over the past few months.*

*Matthew has produced an honors thesis of the highest quality that may be directly applied to making predictions of future market risk, while correcting for biases caused by investor psychology. It is a fascinating result, especially in light of current events in financial markets. I believe that Matt also has a great potential, and I will not be surprised to see him produce high quality research in finance in the future.*

## A COMPARATIVE STUDY OF HIGH-LINOLEIC ACID VEGETABLE OILS FOR THE PRODUCTION OF CONJUGATED LINOLEIC ACID

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Department of Chemistry and Biochemistry

Faculty Mentor: Andrew Proctor

Department of Food Science

### Abstract

Conjugated linoleic acid (CLA) has anti-carcinogenic, anti-diabetic, and anti-atherogenic properties and is present in very small quantities in dairy and beef products. Obtaining optimum dietary CLA levels from these sources requires an undesirable increased intake of saturated fat. A 20% CLA soy oil has been produced by UV photoisomerization of linoleic acid (LA) in soy oil, which is naturally low in saturated fat. However, no other high LA vegetable oils have been studied for their potential as CLA-rich oils. The objectives of this research were to: 1) compare flax, sunflower, corn, soy, and high LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale UV photoirradiation processing equipment, and 2) compare the oxidative stabilities of laboratory-scale processed oils. Seven hundred g of each oil was irradiated with 0.15% iodine catalyst on a laboratory-scale for 168 hours. Oil fatty acid analysis was done before and after processing as fatty acid methyl esters by gas chromatography-flame ionization detection (GC-FID) analysis. Oxidative stabilities of laboratory-scale processed oils were measured gravimetrically for up to 24 days at 64°C. High LA safflower oil produced the most CLA; soy oil produced slightly less followed by corn, with flax producing very little and sunflower none at all. Low CLA yields were due to carotenoids and lipid oxidation in flax oil and carotenoids and turbidity in sunflower oil. The results show that high LA oils should be highly refined before they are used for CLA production. There was no significant difference between the oxidative stabilities of high LA safflower oil and soy oil before or after irradiation, indicating that these oils are the most suitable for high-CLA production, although high LA safflower oil is more expensive.

### Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (octadecadienoic fatty acid) found naturally in dairy and beef products. The most common CLA isomers are *cis*-9,*trans*-11-octadecadienoic acid and *trans*-10,*cis*-12-octadecadienoic acid (Lawson *et al.* 2001), but other isomers are also present in food, including *trans*-9,*trans*-11 and *trans*-10,*trans*-12 (Grinari and Bauman 1999).

Studies indicate that CLA plays anti-diabetic, anti-obesity, and anti-atherogenic roles (McGuire and McGuire 1999; Khanal and Olson 2004). Despite its nutritional benefits, CLA appears naturally at levels of 0.3 – 0.8% of the fat in beef and dairy products, making it difficult to consume the

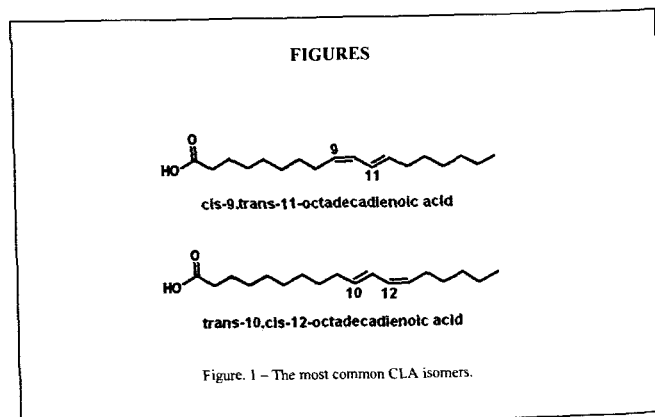


Figure 1 – The most common CLA isomers.

recommended 3 g of CLA per day (Ip *et al.* 1994; Ma *et al.* 1999) that is necessary to produce the desired physiological effects without consuming undesirable amounts of saturated fats and cholesterol. For this reason, an alternative source that contains high levels of CLA but is low in saturated fat and cholesterol would be an excellent addition to the human diet.

CLA can be produced by several means, including biosynthesis through fermentation by anaerobic rumen bacteria (Kepler *et al.* 1966) in bovines. CLA has also been produced by controlled fermentation (Martin and Jenkins 2002; Lee *et al.* 2003; Vahvaselka *et al.* 2004; Lin *et al.* 2005) and organic synthesis (Yang and Liu 2004), but these methods are complicated and time-consuming.

Photoisomerization of linoleic acid (LA) in soy oil has been demonstrated to be a simple and effective alternative method of CLA synthesis (Gangidi and Proctor 2004) that can be completed on a laboratory scale (Jain and Proctor 2006) using a UV/visible lamp with 0.15% iodine as a catalyst with standard photo-chemical irradiation apparatus. However, this method is very time-consuming, requiring 144 hours to synthesize 20% CLA. A pilot-scale method has been developed and optimized by Jain *et al.* (2008a) so that CLA-rich soy oil can be produced on a pilot scale yielding 20% CLA in 12 hours. This requires 0.35% iodine catalyst and a customized illuminated laminar flow unit (ILFU) consisting of borosilicate glass plates and three 450W UV/visible lamps, to increase the oil's exposure to ultraviolet light. This photoisomerization method produces CLA in a food source, whereas previous methods, such as that by Yang and Liu (2004), produced

CLA as an inedible free fatty acid. Only soy oil has been used to study photoisomerization of oil LA, but other high LA vegetable oils, such as flax, sunflower, corn, and high LA safflower oils are available. However, they have not been evaluated as a source of CLA-rich oil.

Oxidative stability is a vital oil quality parameter describing an oil's resistance to rancidity. Lipid oxidation is due to the formation and subsequent degradation of lipid hydroperoxides, which lead to rancid food products as well as the formation of hazardous free radicals (Kanner and Rosenthal 1992). These free radicals have been linked with cancer, atherosclerosis, hypertension, senile dementia in Alzheimer's, and various forms of amyloidosis (Harman 1984). Oils that have a high oxidative stability are superior in quality, safer for human consumption, and more palatable. Currently, there have only been studies on the oxidative stability of CLA-rich soy oil. If other high-linoleic acid vegetable oils can be converted to CLA-rich products via photoisomerization of linoleic acid, characterizing their oxidative stabilities will be key to determining their usefulness as a food product.

Various minor components of vegetable oils will have a significant impact on soy oil CLA yields and oxidative stability, and the amount of these various minor components decreases as degree of processing increases (Jain, et al. 2008b). The reason for refining vegetable oils is to remove substances that reduce oil quality (Jawad 1983). The steps of refinement are degumming, bleaching, winterizing, steam distillation, neutralizing, and deodorizing (Hoffman 1989). Minor components that are removed in these stages include tocopherols, pigments (especially carotenoids like lutein), and phospholipids (Lindley 1998).

**Oil Tocopherols:** The tocopherol content of oils is dependent on plant genotype, climate conditions, and processing and storage conditions (Rabascall and Riera 1987). Tocopherols are found in vegetable oils in four different forms:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols. Very low concentrations of tocopherols are sufficient to protect vegetable oils from oxidation. Usually one tocopherol molecule can protect between  $10^3$  and  $10^8$  polyunsaturated fatty acid molecules (Kamal-Eldin et al. 1996). The method through which tocopherols prevent oxidation involves the transfer of a hydrogen radical from the 6<sup>th</sup> hydroxyl group of the tocopherol chroman ring to the lipid peroxy radical producing a lipid hydroperoxide and a tocopheroxy radical. The tocopheroxy radical is more stable than the lipid peroxy radical due to resonance stabilization, which causes the rate of oil oxidation to decrease in the propagation stage of autoxidation (Choe and Min 2006). The free radical scavenging activity is the highest in  $\alpha$ -tocopherol, making it the most potent of all the tocopherol antioxidants, followed by  $\gamma$ -,  $\beta$ -, and  $\delta$ -tocopherol (Ikeda and Fukuzumi 1977; Chu and Lin 1993). These natural antioxidants are removed during each step of the oil refining process (Jung et al. 1989; Kellens 1997; Alpaslan et al. 2001). It is also known that the various refining steps may affect oxidative

stability of vegetable oils (Jung et al. 1989; Yoon and Kim 1994). The average loss of total tocopherol content during the refining process was found to be greater than 30% (Tasan and Demirci 2004).

**Oil Pigments:** Pigments present in vegetable oils include carotenoids (both  $\alpha$ -,  $\beta$ -, and  $\gamma$ -carotenes and xanthophylls), chlorophyll, diketones, and browning products (Cowan 1976). Carotenoid pigments, such as lutein, shown in Figure 3, are known for their ability to protect against oxidation by absorbing light and preventing photooxidation (Choe and Min 2006).

Vegetable oil colors are measured commercially in terms of yellow, red, blue, and neutral units by the Lovibond® Tintometry RYBN Scale (AOCS Method Cc 13e-92). This technique compares the color of light transmitted through the oil and its cuvette with the color of light transmitted from the same light source through a set of standard glass slides of various colors (Estey 1935). Pigments found in vegetable oils are reduced by the bleaching stage, which uses bleaching clays under low pressure and high temperature (Cowan 1976; Mounts and Khym 1980; Subramanian et al. 1998).

**Oil Turbidity:** Turbidity is a measure of cloudiness in a liquid. Oil components that contribute to turbidity include saturated triglycerides, waxes, free fatty acids, hydrocarbons, and sterols (Popov et al. 1970; Morrison and Robertson 1975; Caupeil 1977). These components are present in refined, bleached, and deodorized (RBD) oils in ppm and ppb concentrations, making their removal difficult (Morrison and Robertson 1975). Studies on vegetable oil turbidity particularly implicate sunflower oil mainly due not only to its wax content but also its saturated fatty acids, especially stearic and palmitic acid (Leibovitz and Ruckenstein 1981). The wax content in sunflower oil decreases with degree of processing (Leibovitz and Ruckenstein 1984) and may vary with seed variety, geographical growing area, and seasonal growing conditions (Moulton 1988). Turbidity can be determined by measuring absorbance at 600 nm (Przybylski et al. 1993).

**Oil Phospholipids:** Phospholipids are a class of lipids in which glycerol is bonded to two fatty acids and a phosphate group which may be attached to an additional chemical group. The classes of phospholipids in soy oil include phosphoglycerides, phosphoinositides, and phytosphingosines (Cowan 1976). The maximum antioxidant activity is observed between 3 and 60 ppm. (Yoon and Min 1987). The removal of these phospholipids will result in the removal of metals like iron and copper, increasing the oxidative stability of the processed oils, though the mechanism of this action is not well understood (Ceri et al. 2000, Reische et al. 2002). Oils cannot be deodorized without oil discoloration until after phospholipid removal (Gutfinger 1978), which occurs by hydration, followed by centrifugation, during the degumming stage of processing (Cowan 1976; Lindley 1998). Degumming reduces the phosphorus content of the oil from 500-900 ppm to 12-170 ppm (Brown and Snyder 1985).

The objectives of this research were to compare:

- 1) Flax, sunflower, corn, soy, and high LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale processing equipment.
- 2) The oxidative stabilities of CLA-rich flax, sunflower, corn, soy, and high LA safflower oils prepared with laboratory-scale processing equipment.

## Experimental Materials and Methods

### Materials

The following vegetable oils were used: High LA safflower (Liberty Vegetable Oil Company, Santa Fe Springs, CA), Soy (Wesson, ConAgra Foods, Inc. Omaha, NE), Corn (Mazola, ACH Food Companies, Inc., Memphis, TN), Sunflower (The Hain Celestial Group, Inc., Melville, NY), and Flax (Spectrum Organic Products, LLC, The Hain Celestial Group, Inc., Melville, NY).

Resublimed iodine (EM Science, Cherry Hill, N.J., U.S.A.) was used as a catalyst for isomerization. Sodium methoxide and anhydrous sodium sulfate (EM Science, Darmstadt, Germany) were used for methyl ester preparation for oil fatty acid analysis by gas chromatography. Commercial heptadecanoic acid (17:0) methyl esters (Sigma-Aldrich, St. Louis, MO, U.S.A.) were used as standards for the GC analysis. Activated carbon was used to remove iodine from the oil samples after photoirradiation and before oxidative stability analysis.

### *Comparison of flax, sunflower, corn, soy, and high LA safflower oils as sources of CLA-rich vegetable oils using laboratory-scale processing equipment*

**Photoisomerization:** A laboratory-scale customized photochemical reaction unit (Ace Glass Inc., Vineland, N.J., USA) and an ultraviolet light source and methodology described by Jain and Proctor (2006) were used. Duplicate 700 g oil samples from each oil source were deaerated by sonication for 10 minutes and then wrapped with aluminum foil to prevent light exposure while being heated to 70°C to dissolve 0.15% iodine. The oil was transferred to the reaction vessel and the photochemical system was connected to a water supply so temperature of the oil could be controlled between 22 and 25°C while being closely monitored with a Traceable Big-Digit Memory Thermometer sensor (VWR International, Friendswoods, TX). The photochemical reaction system was placed on a magnetic stirrer to facilitate continuous oil stirring during the 168-hour irradiation. 10mL samples were collected every 24 hours of the irradiation for fatty acid analysis.

**LA and CLA Fatty Acid Analysis by GC-FID:** Oil fatty acid analysis, including the LA and CLA content, was conducted on all duplicate sample oils both before irradiation and after each day of irradiation. Two replicates from each duplicate oil sample were converted to fatty acid methyl esters (FAMES), which were injected in triplicate by an autosampler

CP8400 for GC-FID analysis. This analysis was conducted according to the method of Christie et al. (2001).

To convert each oil sample to methyl esters, each duplicate irradiated oil was weighed into a 25-mL centrifuge tube in duplicate, and 500  $\mu$ L 1% heptadecanoic acid methyl ester (17:0, internal standard), 2 mL toluene, and 4 mL 0.5 M sodium methoxide in methanol were added. The centrifuge tube was heated to 50°C for ten minutes and then cooled for 5 minutes. To inhibit formation of sodium hydroxide, which could hydrolyze methyl esters to free fatty acids, 200  $\mu$ L glacial acetic acid was added to the centrifuge tube. Then 5 mL distilled water was added followed by 5 mL hexane, and the tube was vortexed for 2 minutes. The hexane layer was extracted and dried over anhydrous sodium sulfate in a 7-mL borosilicate glass vial.

Methyl esters were analyzed by GC using a SP 2560 fused silica capillary column (100 m  $\times$  0.25 mm i.d.  $\times$  0.2  $\mu$ m film thickness; Supelco Inc., Bellefonte, PA) with an FID (model 3800, Varian, Walton Creek, CA). Triplicate 2 $\mu$ L samples, prepared in hexane, were injected by an autosampler CP8400 (Varian), and gas chromatograms were collected by Galaxie Chromatography Workstation 1.9.3.2 (Varian). CLA concentrations were calculated by the following equation:

$$\text{isomer concentration} = \frac{[\text{internal standard concentration} \times \text{peak area} \times \text{response factor}]}{\text{internal standard peak area}}$$

**Tocopherol Analysis:** Tocopherol content (including  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols) was determined in duplicate for each sample oil before irradiation using the AOCS Ce 8-89 method (American Oil Chemists' Society, 1998). Standard stock solutions of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$  tocopherols in methanol (10 mg/10 mL) were prepared and the absorbance of the solution was measured at 292 nm to calculate the concentration ( $\mu$ g/mL). Appropriate volumes of the stock solution of the tocopherol standards were mixed to obtain a mixed tocopherol standards working solution and further diluted with hexane to give a solution containing between 1-5  $\mu$ g/mL of each tocopherol. The HPLC analytical column (250 X 4 mm) was packed with microparticulate silica (5  $\mu$ m). The HPLC mobile phase consisting of propan-2-ol in hexane (0.5:99.5, v/v) was pumped throughout the column at a flow rate of 1 mL/min for at least 30 minutes. About 2 g of the homogenized oil sample was weighed accurately into a 25-mL volumetric flask with hexane. Mixed tocopherol standard working solution (20  $\mu$ L) was injected onto the column, and the area of the tocopherol peaks was recorded using an integrator. Then 20 $\mu$ L of the oil sample were injected and the tocopherols present were identified by reference to the chromatograms obtained from the standards. The areas of the tocopherol peaks were recorded. Two determinations each consisting of duplicate injections were conducted for each oil sample.

**Turbidity Analysis:** Duplicate samples from each sample oil were analyzed for turbidity prior to irradiation. Each

sample oil duplicate was diluted 1:10 by volume in hexane and measured by absorption at 600nm.

**Carotenoid Analysis:** Duplicate samples from each sample oil were quantified as lutein, even though other carotenoids were present. Each oil duplicate was diluted 1:10 by volume in hexane and measured by absorption at 445nm, using the method to determine soy lutein content (Proctor and Snyder 1987).

**Lovibond Color Analysis:** The color of each undiluted oil was measured in duplicate before irradiation in terms of yellow, red, blue, and neutral units by the Lovibond® Tintometry RYBN Scale (AOCS Method Cc 13e-92, AOCS Method Cc 13j-97). This technique relates the color of light transmitted through the oil and its cuvette with the color of light transmitted from the same light source through a set of glass slides of standard size and color (Estey 1935).

**Statistical Analysis:** Analysis of variance (ANOVA) was conducted on all data using JMP Version 5.0.1 (SAS Inst. Inc., Cary, N.C., U.S.A.). A Student *t* test was used to differentiate mean values, with significance defined at  $P < 0.05$ . Standard deviations were also determined.

#### **Comparison of the oxidative stabilities of CLA-rich flax, sunflower, corn, soy, and high LA safflower oils prepared with laboratory-scale processing equipment**

**Sample Preparation:** The sample oils both before and after laboratory-scale irradiation were adsorption processed with 2% SX-51 carbon (Norit Americas Inc., Marshall, TX, USA) at 100°C for 30 minutes to remove residual iodine. During this time, the beaker filled with oil was wrapped in aluminum foil to prevent light exposure. The carbon-adsorbed oil was then filtered using Whatman GF/A filter paper (VWR International Inc.) to remove the carbon adsorbent.

**Gravimetric Analysis:** The oxidative stability of the irradiated oil and corresponding control was determined by the method of Proctor and Bowen (1996). Triplicate 500 mg samples from each duplicate sample were stored at 64°C and weighed daily for up to 24 days, depending on the oil's rate of oxidation. Data are expressed as percent weight change, indicative of the formation and subsequent degradation of lipid hydroperoxides, the primary product of oxidation that leads to rancid products and hazardous free radical formation (Kanner and Rosenihal 1992).

**Statistical Analysis:** Analysis of variance (ANOVA) was conducted on all data using JMP Version 5.0.1 (SAS Inst. Inc., Cary, N.C., U.S.A.). A Student *t* test was used to differentiate mean values, with significance defined at  $P < 0.05$ . Standard deviations were also determined.

## **Results and Discussion**

### **Comparison of oils as sources of CLA-rich vegetable oils**

**LA and CLA Fatty Acid Analysis by GC-FID:** High LA safflower oil had the highest initial LA, while soy and corn oils had intermediate levels (Table 1). Sunflower oil had less

LA while flax oil had the least. Oils with the highest initial LA produced the most CLA. However, the soy conversion rates were lower than those reported by Jain and Proctor (2006). This may be due to using a different soy oil source that contained different minor component levels, which are known to reduce CLA yields (Jain, et al. 2008b). Corn and soy oils had similar initial LA contents, but corn oil produced less CLA.

Flax oil produced very little CLA with 14% initial LA and sunflower oil produced no CLA at all, despite having 34% initial LA. The conversion of LA to CLA in each oil was: ~23% in high LA safflower oil, ~20% in soy oil, ~11% in corn oil, ~5% in flax oil, and 0% in sunflower oil. Differences may be due to increasing minor component levels in each oil, which

Table 1. Initial and final linoleic acid values and final CLA values after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.

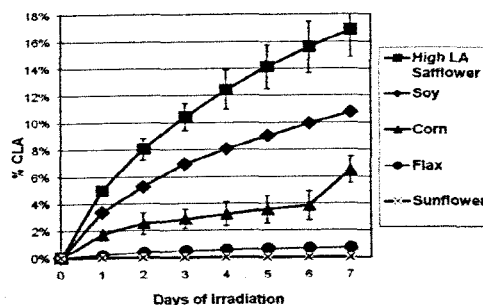
Oil	Initial Linoleic Acid	Final Linoleic Acid	Final CLA
Flax	14.7% ± 0.1 a	14.7% ± 0.1 a	0.7% ± 0.1 a
Sunflower	34.0% ± 0.4 b	33.9% ± 0.4 b	0.0% ± 0.0 a
Corn	57.5% ± 1.1 c	49.5% ± 0.6 c	6.5% ± 1.0 b
Soy	53.7% ± 0.3 d	42.9% ± 0.4 d	10.8% ± 0.1 d
High LA Safflower	72.6% ± 0.2 e	54.9% ± 2.2 e	16.9% ± 2.0 d

\*Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ .

have been shown to reduce CLA yields (Jain et al. 2008b). A comparison of soy and corn CLA yields and sunflower and flax CLA yields show that LA levels alone are not good predictors of CLA production. The highest rate of CLA production for each oil was during the first day of irradiation (Figure 2) and was followed by a decrease in the rate of CLA production each subsequent day.

In oils with the most initial LA, the amount of total CLA produced continues to increase after seven days of irradiation, indicating the benefit of further irradiation for high LA safflower and soy oils. The rate of CLA formation in corn oil was less than that of soy oil despite having slightly more initial LA. In addition, flax and sunflower oils did not produce

Figure 2. CLA production in high LA safflower, soy, corn, flax, and sunflower oils by laboratory-scale processing.



a significant amount of CLA despite the presence of LA. This further suggests that factors other than initial LA may affect CLA yields.

The *trans*-,*trans*- isomers CLA isomers (Table 2) were produced in the greatest yield for all irradiated oils, comprising 70% of the total CLA.

The remaining isomers were a mixture of *cis*-,*trans*- (9,11 and 10,12) and *trans*-,*cis*- isomers (9,11 and 10,12), which were present in greater quantities than found in animal Table 2. CLA isomer levels after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.

Table 2. CLA isomer levels after photoirradiation for seven days on the laboratory-scale processing equipment as measured by GC-FID.

Oil	cis-9,trans-11	trans-9,cis-11 and cis-10,trans-12	trans-10,cis-12	trans-,trans- (8,10, 9,11, and 10,12)
Flax	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a	0.7% ± 0.1 b
Sunflower	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a	0.0% ± 0.0 a
Corn	0.5% ± 0.5 a,b	0.5% ± 0.4 a	0.6% ± 0.3 b	4.7% ± 0.2 b
Soy	1.1% ± 0.0 b,c	1.3% ± 0.0 b	1.1% ± 0.0 c	7.3% ± 0.1 b
High LA Safflower	1.6% ± 0.1 c	2.0% ± 0.3 b	1.6% ± 0.1 d	11.8% ± 1.9 c

\*Data with the same letter within the same column are not significantly different, with significance defined at  $P < 0.05$ .

products (Jain and Proctor 2007a). The large yield of *trans*-,*trans*- isomers differs from the results of the biosynthesis and bacterial fermentation methods of CLA formation, which mainly produce the *cis*-9,*trans*-11 isomer. However, the *trans*-,*trans* CLA isomer still leads to CLA's health benefits (Gavino et al. 2003; Kuan-Lin et al. 2005) and is derived from *cis*-,*trans*- CLA isomers. It is probably favored during photoisomerization because of the greater stability of the *trans*- isomer relative to the *cis*- isomer (Jain and Proctor 2007b).

High LA safflower, soy, corn, and sunflower oils were found to have a total tocopherol content in excess of 100 ppm, with no significant difference (data not shown). Flax oil had a significantly lower tocopherol concentration of less than 40 ppm. For high LA safflower and sunflower oils,  $\alpha$ -tocopherols were the most prominent, while  $\beta/\gamma$ -tocopherols were the most common in soy, flax, and sunflower oils.  $\delta$ -tocopherols only reached significant levels in soy oil. The  $\delta$ -tocopherols and  $\gamma$ -tocopherols are the most potent radical scavenging antioxidants of all tocopherols (Ikeda and Fukuzumi 1977; Chu and Lin 1993), and may therefore hinder the free radical mechanism through which LA is isomerized to CLA. Since  $\delta$ -tocopherols were present in soy oil at high levels, this may explain why CLA yield in soy oil was lower in this study than in previous studies (Jain and Proctor 2006, 2008a). It is difficult to know which oils have the highest  $\gamma$ -tocopherol content, because  $\gamma$ -tocopherols and  $\beta$ -tocopherols co-elute during HPLC analysis. It can be reported that the oils with the highest  $\beta/\gamma$ -tocopherol content were soy oil and corn oil, with ~70 ppm, whereas the other oils studied were all below 30ppm of  $\beta/\gamma$ -tocopherols. This may explain why both soy and

corn oils yielded less CLA than expected from their initial LA contents.

**Turbidity Analysis:** Turbidity was only measurable in sunflower oil with an absorption of  $0.60 \pm 0.00$  at 600nm (data not shown). Sunflower oil is the primary vegetable oil noted for its turbidity after oil refining (Leibovitz and Ruckenstein 1981, Leibovitz and Ruckenstein 1984). Turbidity is probably due to residual waxes and sterols, which may interfere with UV light absorption by LA. Although turbidity is low, it may explain why no CLA was produced from sunflower oil. "Winterizing" the oil by storing at 4°C will cause precipitation of the waxes and sterols and is commonly done with soy oil.

**Carotenoid Analysis:** The only vegetable oils to show a significant lutein concentration were flax oil, with 9.8 ppm lutein, and sunflower oil, with 1.2 ppm lutein, both of which produced little to no CLA. High LA safflower, soy, and corn oils had no measurable lutein content and produced the most CLA. The significant amount of carotenoids in flax oil may have hindered CLA formation due to UV absorbance (Choe and Min 2006), possibly contributing to flax oil's low conversion rate (~5%) of LA to CLA.

**Phospholipid Phosphorous Analysis:** Phosphorous was not detected in high LA safflower, soy, corn, or sunflower oils at any level but was detected in flax oil at 5.79 ppm and is within the range in which phospholipids provide antioxidant activity (Yoon and Min 1987). The data indicate a very high degree of processing in terms of degumming, which reduces the oil phospholipid phosphorous content from 500-900 ppm to 12-170 ppm (Brown and Snyder 1985). Phospholipids sometimes cause turbidity, but this was not an issue in this study.

#### Comparison of the oxidative stabilities of CLA-rich

**Gravimetric Analysis:** Table 3 summarizes the induction times for each oil's oxidation before irradiation, after irradiation, and after activated carbon treatment to remove residual iodine. Unirradiated corn and sunflower oils each had the longest induction time of all the oils without significant difference, indicating high oxidative stability, probably due to high tocopherol content.

Unirradiated high LA safflower and soy oils each had intermediate induction times without significant difference, Table 3. Oxidation induction times (days) of flax, sunflower, corn, soy, and high LA safflower oils determined gravimetrically after being processed with laboratory-scale equipment and incubated at 64°C.

Table 3. Oxidation induction times (days) of flax, sunflower, corn, soy, and high LA safflower oils determined gravimetrically after being processed with laboratory-scale equipment and incubated at 64°C.

Oil	Unirradiated	Irradiated, without iodine adsorption	Irradiated, with iodine adsorption on carbon
Flax	1.5 ± 0.7 a,b,c	1.0 ± 0.0 a,b	0.0 ± 0.0 a
Sunflower	12.0 ± 1.4 g	8.0 ± 0.0 f	0.5 ± 0.7 a
Corn	12.0 ± 1.4 g	7.0 ± 2.8 e,f	1.0 ± 1.4 a,b,c
Soy	7.0 ± 1.4 e,f	5.5 ± 0.7 d,e	3.0 ± 0.0 b,c
High LA Safflower	6.0 ± 0.0 e	3.5 ± 0.7 c,d	2.0 ± 1.4 a,b,c

\*Data with the same letter are not significantly different, with significance defined at  $P < 0.05$ .



also probably due to high tocopherol content. Unirradiated flax oil had a significantly shorter induction time than the other four oils, indicating the lowest oxidative stability. This is probably due to its low tocopherol content and its high (60%) linolenic acid ( $C_{18:3}$ ) content, as determined by GC-FID. Linolenic acid is two times more prone to oxidation than linoleic acid and resulting conjugated dienes absorb in the UV region. Tokle et al. 2009 (in review) also showed that a small degree of lipid oxidation dramatically reduces soy oil CLA yields by studying the effect of various peroxide concentrations on CLA production. Therefore, the oxidative status of the oils may be responsible for their lower yields.

Irradiated sunflower, corn, and high LA safflower oils without adsorption treatment had a decrease in induction times compared to the unirradiated oils. High LA safflower and corn oils had the largest significant decreases in induction time, followed by sunflower oil (33%). Irradiated flax and soy oils did not show a significant difference in induction time from unirradiated flax and soy oils. This may be due to flax oil's high lutein concentration of 9.8 ppm, since lutein prevents photooxidation, and soy oil's high level of  $\delta$ -tocopherols of 9.7 ppm, which are very potent antioxidants (Estey 1935).

After irradiation and activated carbon treatment, sunflower, corn, and soy oils showed significant decreases in induction time, indicating that while the carbon adsorption process was meant to remove iodine, it also may remove antioxidants like tocopherols and lutein from the oils, increasing the likelihood of oxidation. Though flax oil did not show a significant decrease in induction time following this process, it was probably less oxidatively stable relative to the other sample oils before irradiation. High LA safflower oil did not have a significant decrease in induction time following the activated carbon treatment, which may indicate that there was still significant residual tocopherol antioxidant capacity after adsorption.

## Conclusion

Soy oil and high linoleic acid safflower oils were the only viable candidates for the production of CLA-rich vegetable oils. Since high LA safflower oil is more expensive than soy oil, soy oil is more commercially viable for CLA production. The majority of CLA produced in all the oils was as *trans-trans*- isomers, due to their greater stability. A high linoleic acid vegetable oil is important in producing a CLA-rich product, but initial LA content is not the only important factor. Factors such as carotenoid content, conjugated diene oxidation products, and turbidity reduce CLA yields. Sunflower oil did not produce any CLA after laboratory-scale processing despite containing appreciable levels of LA, probably due to oil turbidity reducing light penetration. Flax oil produced <1% CLA after laboratory-scale processing, due to its relatively low LA level, high carotenoid content, and low oxidative stability resulting from its high linolenic acid content. Oils should be high in linoleic acid, highly refined, and minimally oxidized before they can be used for CLA production.

Oxidative stability of the oils before irradiation was protected with significant tocopherol levels, with the exception of flax oil which was less stable. There was a loss of oxidative stability after processing, probably due to the presence of iodine free radicals. Iodine removal by carbon adsorption did not stabilize the oils, possibly because of simultaneous adsorption of tocopherols. The low CLA yields and oxidative stability of flax oil in this study may indicate that the presence of lipid oxidation products prior to irradiation negatively impact CLA yields. Flax oil's initial linolenic acid ( $C_{18:3}$ ) content was 60%, which also negatively impacts its oxidative stability and makes it unlikely that high CLA yields will result from the low initial amount of initial LA.

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#### Mentor Comments:

Andrew Proctor details the process Whitney Gammill went through to develop and complete her own unique research project and the importance of the results of her study.

*I met Whitney in the Fall of 2006 when she was a student in the 'Food Science Orientation' class. I gave a lecture in this course regarding my research on conjugated linoleic acid (CLA) rich soy oil production by isomerizing soy oil linoleic acid. She then approached me regarding the possibility of working with me on her Honors thesis and began working with me on her project. I was immediately impressed by her conscientious attitude and outstanding academic record. She is a very serious, determined student who has clear academic and career goals. Whitney showed herself to be a gifted laboratory worker and highly motivated to conduct research. She subsequently evaluated the potential of various linoleic acid containing oils to produce CLA, which have significant health benefits.*

*Whitney quickly learned our techniques to photo-isomerize oil linoleic acid to CLA. She also rapidly developed the skills necessary to conduct oil fatty acid analysis by conversion of fatty acids to methyl esters, which she quantifies by gas chromatography. She confidently used our novel photo-isomerization vegetable oil processing techniques and related lipid analytical methods to contribute new findings to the growing field of CLA studies. Whitney has made more progress than I anticipated in the time frame available, while working in the laboratory with minimum supervision.*

*Whitney has the academic performance, laboratory skills and personal qualities to enable her to succeed as a researcher. She has the ability to comprehend important concepts rapidly and ask crucial questions necessary to pursue a line of inquiry. Her thorough understanding of chemistry and the related sciences and her desire to understand the theoretical basis*

*of the research project is essential to produce quality work and evidence of her attitude and potential to make a serious impact with her future work. She also has a remarkable ability to focus on a task and follow it through to completion without succumbing to discouragement along the way. Her willingness to do laboratory work primarily during nights and weekends, without being requested to do so, is one example of her determination to achieve research success. I believe her tenacity and maturity will serve her well in the future and deserves the recognition of this award. Although the research is part of an ongoing research program on CLA production, Whitney has very much made this her own project and developed the knowledge, understanding and expertise to effectively communicate the significance of the research and her findings.*

*This paper is an abbreviated version of her Honors thesis. Whitney's research is significant and timely since dietary increase in CLA is related to reducing obesity and obesity related diseases. The importance of her work was recognized in 2008 by the American Oil Chemists Society (AOCS), when she presented her studies at the AOCS Annual meeting. The AOCS Health and Nutrition Division awarded her first prize in the poster competition where she was competing against graduate student researchers from throughout the world. She was also awarded the prestigious Barry M Goldwater Scholarship in 2008.*

*The impact of this investigation was enhanced this year by a recent study conducted by Dr. Devareddy at UofA that showed that 1% dietary CLA-rich oil, from our laboratory, decreased the serum cholesterol of obese rats by 38%. This highlights the nutritional and health importance of CLA-rich oil production and will be an important stimulus to commercialization.*

*The CLA-rich soy oil production process was patented in 2008 and a new company, 'Ultra V Technologies' formed to bring the process to commercialization. Whitney's findings will be important in selecting oils for CLA production and determining to what degree they should be refined.*

## A COMPUTATIONAL MODEL OF BREAST DUCTS

By Jordan Greenlee

Department of Electrical Engineering

Faculty Mentor: Magda El-Shenawee

Department of Electrical Engineering

### Abstract

*Ductal Carcinoma represents almost 75% of breast cancer. In this specific type of cancer, malignant cells in the breast ducts invade the surrounding healthy tissue. Almost all researchers who are investigating microwave modality in the area of breast cancer detection employ oversimplified models of the internal structure of the breast. Use of engineered or biologically inaccurate models can render inaccurate results. Therefore, a mathematical biological model was implemented in this work aiming to bridge the gap between physiologists and engineers.*

*The results show that the proposed breast duct model has the capability of modeling the breast ducts of different kinds of women. The simulated breast ducts of older women have smaller breast ducts, leading to less dense breasts, which is in agreement with medical knowledge. Also, younger women have larger breast ducts leading to more dense breasts, which are consistent with the obtained results. The model was implemented computationally using the computer language C++ in both two and three dimensions. The potential impact of the research is to provide researchers with a greater understanding of the breast ducts as cancer treatment models evolve.*

### I. Introduction

There are current efforts that utilize simulated mammary gland models for theoretical research [1], [2]. To do this, it is necessary to recreate a mammary gland that is consistent biologically. Otherwise, research outcomes using simulated breast models will be inaccurate. Figure 1 shows a sample breast similar to one found in a paper [2] by a researcher using microwave methods to detect breast cancer. A simple look into the most basic anatomy books or websites shows that the model illustrated in Figure 1 is too much of an oversimplification. Random cylinders and spheres attempting to explain the complex morphology of the breast ducts is archaic.

Breast ducts employ branching morphogenesis during growth (see Figure 2). A single duct grows from the nipple for three weeks postpartum. Three to four weeks postpartum, the ovarian function begins, which leads to an increase in end bud elongation and branching. The end buds then extend and branch to form the breast ducts.

The ducts of the breast form by branching morphogenesis similar to the vasculature and lungs. There are models [3]

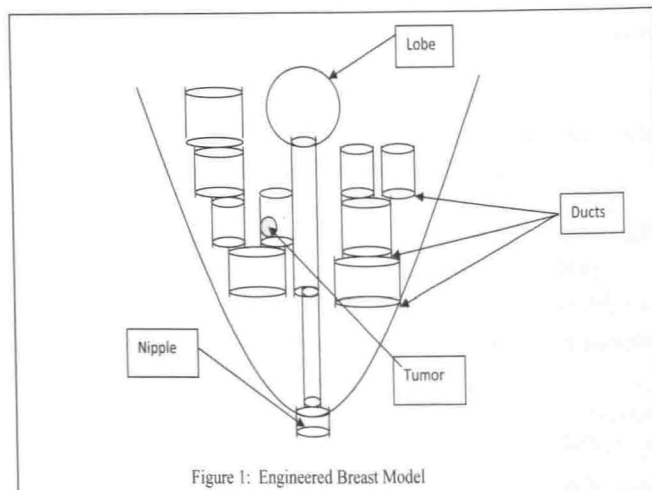


Figure 1: Engineered Breast Model

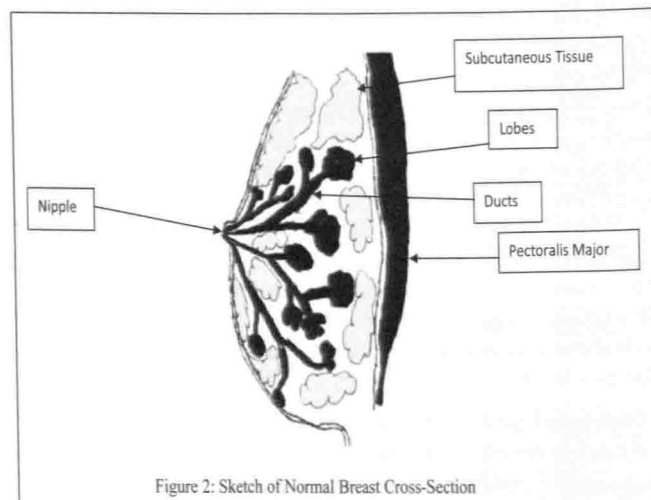


Figure 2: Sketch of Normal Breast Cross-Section

that describe this branching morphology, and it has been determined biologically that these structures can be modeled using a branching tree where a mother branch bifurcates into two or more smaller branches, resulting in a network spanning an entire cavity.

Unfortunately, the simplicity of this model is inhibiting, and its use will result in a biologically skewed breast duct model. In an extensive study [4], 72 breasts were excised for cancer and analyzed for the number of ducts. The researchers determined that the nipples contained 11 - 48 ducts with a median of 27 central ducts. Interestingly, this study found that "half of the breast was drained by three ducts and 75% by the largest six. Conversely, eight small duct systems 39

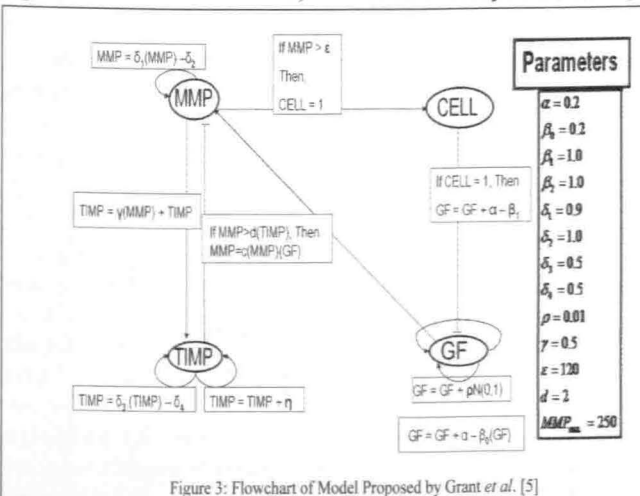
together accounted for only 1.6% of breast volume" [4]. This finding establishes the heterogeneity of the breast duct system. Branching tree algorithms are inconsistent with actual breast ducts because they do not take into account how real breast ducts grow. They attempt to recreate the end-product by ignoring the means by which the breast ducts achieve the intricate patterns present.

However, there is present research [5] that takes into account biological mechanisms in modeling the breast ducts. This model functions on the basis that breast ducts elongate and bifurcate as a result of the extracellular matrix breaking down. There is an activator (matrix metalloproteinases) and an inhibitor (tissue inhibitors of metalloproteinases) present in this model, and these matrix metalloproteinases serve to break down the extracellular matrix, mainly the collagen and laminin. This model serves as the starting point for this study, which further developed a model that would more closely simulate the actual internal structure of the breast.

## II. Methods

The model in this research was developed based on two activator-inhibitor reactions that have been experimentally established by researchers to occur naturally in the breast. The first reaction occurred between matrix metalloproteinases and tissue inhibitors of metalloproteinases. It was implemented in a previous model as illustrated in Figure 3 [5].

If the matrix metalloproteinases (MMP) reached a certain value at a point in the domain, then that point became a breast duct cell. If there was a breast duct cell at a certain point in the matrix, then that cell inhibited the proliferation of growth factors by inhibiting growth factor in that specific cell, which was then averaged over a Moore Neighborhood of three. However, growth factor was an activator of MMP. Therefore, if a point was a breast duct cell, it would eventually be inhibiting



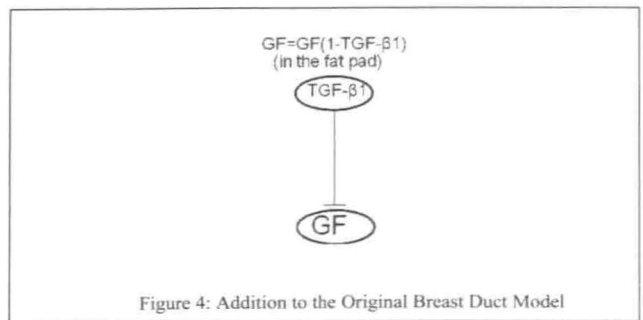
MMP, which in turn inhibited the creation of new breast duct cells. This prevented anastomoses in the model. MMP also was an activator of tissue inhibitors of metalloproteinases (TIMP) which was an inhibitor of MMP. Thus, there was an element of

feedback in the system. There was a random number between zero and one that was added to Growth Factor and allowed for variation in the system.

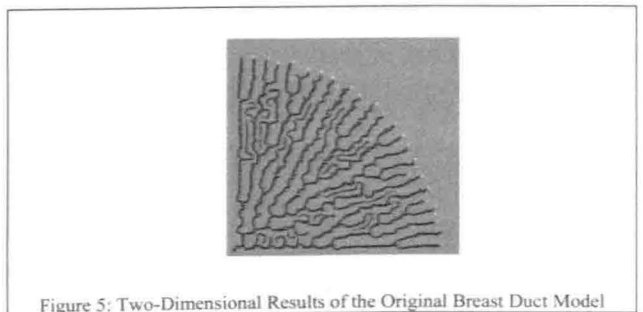
The second reaction occurred between growth factors and transforming growth factor – beta1 (TGF-β1). The work in [6] states, “Excellent evidence that TGF-β1 (Transforming Growth Factor) naturally inhibits this infilling, possibly by blocking hepatocyte growth factor synthesis.” The fat pad can synthesize hepatocyte growth factor. Making use of this finding, an addition was made to the model proposed by Grant et al [5] shown in Figure 4. In the fat pad, growth factor was inhibited by transforming growth factor – beta one as proposed by Silberstein [6].

The motivation for this addition to the model came about in implementing a three-dimensional version. In the two-dimensional model, the duct cells tended to grow on boundaries of the available space as shown in Figure 5.

The breast duct cells congregating on the boundaries did not appear to be problematic in the two-dimensional model, but when the model was brought into three dimensions (Figure



6), it was apparent that an addition to the original model was needed. In the first attempt at a three-dimensional model, the parameters were restricted to a conical area with closed boundaries as originally proposed by Grant et al [5].



## III. Results

The first attempt at a three-dimensional model obviously conflicted with the requirement that the model be biologically accurate. It was then that the boundary conditions were determined to be problematic.

To understand why the boundary conditions were problematic, it is first necessary to understand the way in

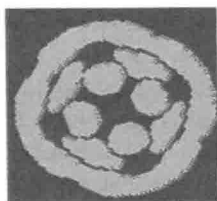


Figure 6: Cross-Section of Original Model in Three-Dimensions

which the activators and inhibitors travel in the simulation. The simulation can be considered as a complex cellular automaton. A cellular automaton is a method used to model biological structures as well as structures in other fields of study [7]. It is a discrete model, so a grid is established and each point in the grid is often considered a cell. These cells then interact with each other and other nutrients and proteins in the system to create a macroscopic order that mimics that found in-vivo.

In cellular automata, the cells in the grid can interact in two common ways. The first, which is employed in the breast duct model, is called the Moore neighborhood. Because it is actually used in the breast duct model, the Moore neighborhood is visited in greater detail while describing it. In a Moore neighborhood, a cell is averaged within a certain neighborhood or radius. All of the cells within the radius are added together and then that sum is divided by the total number of cells in the neighborhood. In Figure 7 (below), a Moore neighborhood is illustrated in a  $10 \times 10$  grid with a cell denoted by "x". An average over all of the neighboring cells (dark pixels) and the cell itself is conducted. This is a two-dimensional example of a Moore neighborhood with radius=2.

The other most common neighborhood in cellular automata is the von Neumann neighborhood. Whereas the Moore neighborhood is the set of all cells that one could walk to if one could walk cardinal or ordinal directions, the von Neumann neighborhood is the set of cells one could travel to by only traversing cardinally. An example of a von Neumann neighborhood with a radius of two is shown in Figure 8 (above). The set of dark squares are those included in the

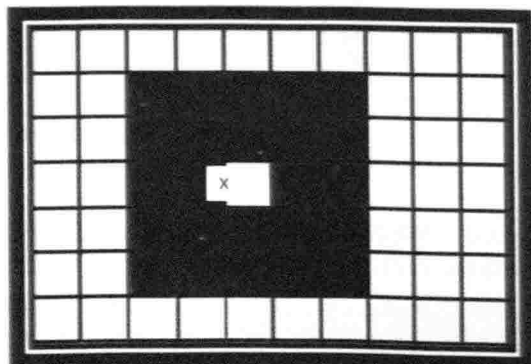


Figure 7: An example of a Moore's neighborhood

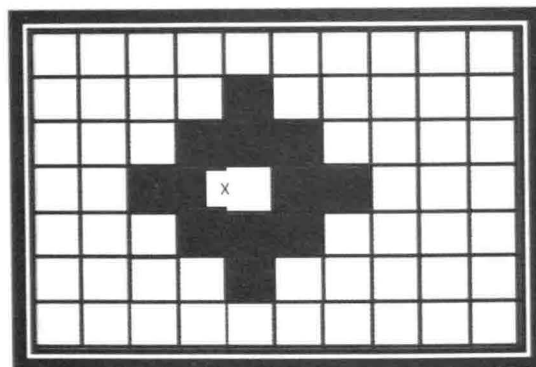


Figure 8: An example of a von Neumann neighborhood

neighborhood for the cell with an "x" in it.

The conical area in Figure 6 creates problems because the Moore neighborhood was being averaged over fewer cells at the edge of this conical boundary. Because it was being averaged over fewer cells, the levels of MMP tended to be higher. Thus, vast amounts of duct cells were being formed on the edge of the boundaries which led to a biologically inaccurate result.

Silberstein [6] provided biological insight into what inhibited the breast duct from growing at the edge of the fat pad. These findings were then implemented in the simulation. A fat pad was created in the simulation, and three breast duct system seeds, which were large amounts of MMP at different intervals in the simulation, were planted. The odd intervals were implemented in order to investigate the interactions between the duct systems at different intervals as shown in Figure 9.

Multiple breast duct systems were not implemented in previous work [5]. Only with the new boundary conditions would multiple breast duct systems result in a meaningful pattern, so the biological accuracy of the new configuration could be determined.

Figure 10 illustrates results from the three-dimensional breast duct model. The level of transforming growth factor

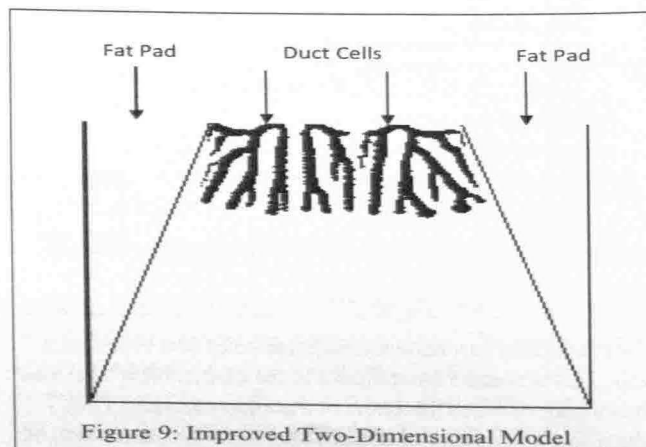


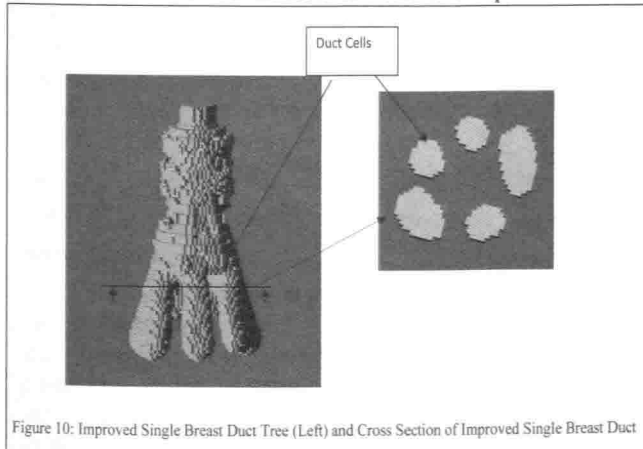
Figure 9: Improved Two-Dimensional Model



– beta one, which inhibits growth factor in the fat pad in the model, was increased from the two-dimensional model. Additionally, the Moore neighborhood for growth factor was increased from three to four. This was needed because the three-dimensional grid used was much larger, i.e.  $300 \times 300 \times 300$  pixels beginning at (0, 0, 0) and ending at (300, 300, 300) in the x, y, and z- directions, respectively. The two-dimensional model required  $300 \times 300$  pixels beginning at (0, 0) and ending at (300, 300) in the x and y directions. Because of this change, growth factor could travel faster with lower levels in the entire grid.

#### IV. Conclusions

Relatively little is known about the breast ducts in three dimensions, mainly due to the way samples from the breast are collected to produce three-dimensional images of the breast [4]. The usual way the three-dimensional breast duct is imaged is by cutting thin slices of a breast and charting the progress of each duct. This method makes it difficult to compare observed



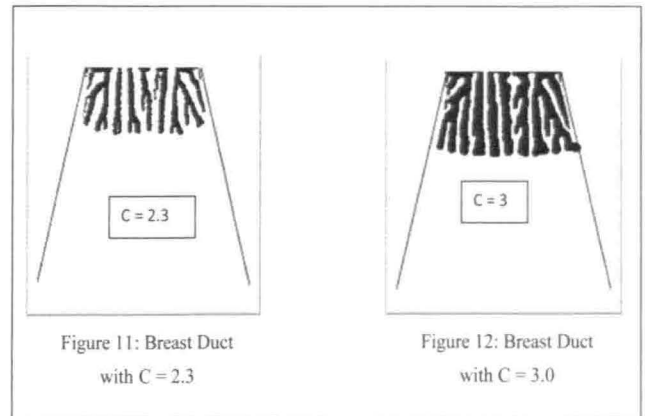
in-vivo sample images with the model in this work.

However, a few key points can be made about the model proposed versus that found in nature. In Silberstein's paper [6], he notes how the breast ducts tend to trail off in nature while approaching the fat pad. This is consistent with the results of the current model, suggesting that it is qualitatively similar to that observed in nature.

Also, by changing different parameters in the model, it was fit to different women. For example, an older woman has smaller breast ducts and less dense breasts. In order to take this into account, the parameter "c" was lowered and a thinner breast duct was formed. Conversely, a younger woman's breast ducts are much thicker and the parameter "c" was increased. If a pregnant woman's ducts were being modeled, c would need to be even bigger. The next Figures (11 and 12) demonstrate this relationship in two examples.

Figure 11 illustrates six breast duct systems of an older woman whose breast ducts have begun to shrink while Figure 12 is a simulation of a woman who is much younger and has much denser breasts. Both figures have six breast duct systems

all starting in the same spots on the grid for both simulations. Also, both figures exhibit branching morphology with breast ducts ending at the fat pad. This is congruent with that seen in-vivo.



The three-dimensional results of the improved breast duct model (Figure 10) were similar to the two-dimensional results. The three-dimensional model results exhibited branching morphogenesis, which is consistent with medical knowledge. The cross-section in Figure 10 was not exactly symmetric due to the slight random element in the model. However, this was a simulation of one single breast duct system. If there were more in the simulation (like Figures 11 and 12), each separate breast duct system would be inhibiting the growth of others around it. This is because breast duct cells inhibit MMP in the cells around them. This inhibition would cause some breast ducts to flourish much less than others.

Additionally, the improved three-dimensional breast duct model no longer had breast duct cells congregating on the edges. This is apparent in the comparison of the cross sections in Figures 10 and 6. While Figure 6 had a ring surrounding the breast ducts, Figure 10 did not exhibit this characteristic. The improvement was due to the addition of the reaction between growth factors and transforming growth factor – beta 1 (TGF- $\beta$ 1). Thus, the biological accuracy of the original model has been improved.

The ability to model the breast ducts in three dimensions will be a useful tool to researchers. Utilizing a biologically accurate breast duct model will render more accurate results and a greater understanding of the breast ducts.

#### Acknowledgments

This work is funded by the National Science Foundation Award Number ECS-0524042, the State Undergraduate Research Fellowship, NSF/IRE Award # ECS-0524042, and the University of Arkansas Honors College.

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#### **Mentor Comments:**

According to Professor Magda El-Shenawee, Jordan Greenlee has developed a model that more accurately represents breast ducts so that cancer detection can be improved in the future. She writes:

*Jordan's major area of study is electrical engineering with major in mathematics. Jordan spent the spring semester of 2007 studying Mathematical Biology and other mathematics courses at the University of Dundee in Scotland.*

*Jordan has demonstrated a potential for mathematical biology research while he was working on modeling the breast. His project dealt with understanding, modeling and simulating the structures of breast ducts and blood vessels. His work is of extreme importance to the 3-year NSF funded (\$250,000) project on breast cancer detection. The outcome of Jordan's research will significantly help the research group to understand the biology of the breast.*

*Almost all researchers who are investigating microwave modality for breast cancer detection model the breast from the engineering point of view, which is inaccurate. Therefore, a mathematical biology based model is needed to bridge the gap between physiologists and engineers. This is the motivation of Jordan's model and paper. His results show that the proposed model has the capability of modeling the breast ducts for young and old women. His results agree, in principle, with the published medical information. Jordan's research provides scientists with a greater understanding of the breast ducts and blood vessels.*



## EVOLUTION OF GENE STRUCTURE IN MULTICELLULAR EUKARYOTES

By Maria Hester

Department of Biological Sciences

Faculty Mentor: Douglas Rhoads

Department of Biological Sciences

### Abstract

*We investigated the patterns of intron conservation in eukaryotes for five different genes. The genes examined were ribosomal proteins L8, S14 and S17, along with elongation factor 2B and triose phosphate isomerase. Intron conservation for S14, S17, and triose phosphate isomerase was determined for 32 species representing the major branches of multicellular eukaryotes. For 25 conserved introns 16 were phase 0, five were phase 1, and four were phase 2. Triose phosphate isomerase had five of nine conserved introns shared between plants and animals, where S14 had one of nine and S17 had one of seven. However, there were two plant S14 introns that could be found in single soil-living organisms from the animal branch, suggestive of horizontal transfer.*

**Supplementary Figures are available at <http://biscweb.uark.edu/drhoads/pubs/IntronsSupFigs.pdf>**

### Introduction

Introns are prevalent in all eukaryotes whose genomes have been fully sequenced, though the densities and sizes of the introns vary greatly (Carmel 2007a). Despite the widespread prevalence of introns, little is known about the origins of introns and what role, if any, they played in gene and genome evolution in eukaryotes (Carmel 2007b). Competing theories have been proposed to address these issues, including the "introns early" and "introns late" theories of intron influence on eukaryote evolution (de Souza 1996). These theories attempt to answer the question of whether introns predate eukaryotes or have been acquired more recently during eukaryotic evolution (Logsdon 2004).

Genes in eukaryotes are not just linear sequences that code for proteins. The gene is recognized as the region that is transcribed to make an initial transcript. The initial transcript is processed in the nucleus to add a poly A tail, and specific regions are precisely removed by a protein-RNA complex called the spliceosome. Those portions that are removed are called introns. The remaining portions that are joined together to form the mature mRNA are called exons. As many as 80% of intron positions are conserved across vastly different eukaryote lineages. The other 20% of introns can either be explained by novel insertions or by precise deletions (Coulombe-Huntington 2007).

The conservation and non-conservation of intron position within genes is where the battle between the two competing theories lies. The "introns early" theory is based on the

notion that introns were present before eukaryotes arose from prokaryotes, perhaps even present in the original genome at the origin of life in the protogenote. Since the divergence of prokaryotes and eukaryotes, prokaryotes and some single-celled eukaryotes have streamlined their genome through loss from intron-rich ancestral genes that predate eukaryotic cells (Logsdon 2004). Past studies have shown that early eukaryotic ancestors were relatively rich in introns (Schwartz 2008). According to the exon shuffling theory, introns were essential components of gene evolution as they can increase recombination of exons as gene fragments (Long 1995). Therefore, exons were used as building blocks in the evolution of eukaryotic organisms to create novel genes. Introns persist in eukaryotes as a result of their role in genomic evolution. This theory proposes that introns exist today because they were used historically as a quicker way to form the diversity of genes that are now present (de Souza 1996). The introns early theory holds that introns, through exon shuffling, facilitated the origin of new proteins through recombination. Therefore, introns were an intrinsic element of the first protein encoding genes (Basu 2008).

Exon shuffling would be an extremely effective method to create a large diversity of protein structures (de Souza 1996). The absence of introns in present day prokaryotes is attributed to the complete loss of introns through "genome streamlining" (Basu 2008). In a study using a large database of eukaryotic genes, it was found that at least 19% of the exons present were the result of exon shuffling, and these exons were often found in the conserved regions of ancient genes that are homologous to prokaryotic genes (Long 1995). This data supports the theory that introns were indeed present in prokaryotes at some point in evolutionary history.

In contrast, the introns late theory holds that prokaryotes never possessed introns; introns and the spliceosome emerged during early eukaryotic evolution (Basu 2008). The current distribution of introns can be explained by processes of both gain and loss (Logsdon 2004). Introns are present in certain organisms because molecular processes introduce them faster than the counterselection, or evolutionary drift, mechanisms can remove them. Therefore, they have limited significance in eukaryote evolution and little, if any, function (de Souza 1996). The introns late theory concludes that there have been recent instances of intron insertion into eukaryote genomes. These introns were inserted into preexisting genes at some point in evolution (Long 1995).

A recent study shows that some eukaryotic lineages may still be gaining introns, while others appear only to be losing them. A comparison of the human, dog, rat, and mouse genomes identifies over a hundred instances of intron loss, but no evidence of intron gain, over the last one hundred million years since these organisms diverged (Coulombe-Huntington 2007). However, a genome-wide study of *Drosophila* shows that there has been recent intron gain within the *Drosophila* lineage, with the latest gain occurring around ten million years ago (Coulombe-Huntington 2007). Therefore, the rates for intron gain and loss differ between specific eukaryotic lines.

Intron phase, or the position of the intron relative to codons in the gene, is theorized to be important in showing which of these theories is most valid. Intron phase can be either 0, 1, or 2 (Long, 1995). According to the intron late hypothesis, introns in each phase should have equal distributions, because addition of the intron to a pre-existing gene would have no effect on the coding function of the gene. Conversely, the introns early theory would suppose that a non-random distribution is more likely, favoring phase 0, because exon shuffling would favor introns in phase 0. If most introns were phase 0, then exon shuffling would not alter the protein sequence encoded by the exon (Long, 1995). Phase 0 introns occur between two codons. If the introns early theory is correct, introns should occur in this phase because ancient exons would have been independent units, and phase 0 introns would not have interfered with coding structure of exons after shuffling. A survey of a subset of 296 genes identified 1496 introns where 55% were phase 0 introns, 24% phase 1 introns, and 21% phase 2 introns. This nonrandom distribution of intron phase supports the introns early theory (Long 1995).

A recent study has shown that there may be three distinct modes of evolution of intron/exon structure (Carmel 2007b). The first mode is the primary, balanced mode that operates in all lineages. In this mode, intron gain and loss are strongly and positively correlated. The second mode is one of an elevated rate of intron loss. This mode is prevalent only in certain lineages, such as insects and fungi. The third mode highlights an elevated rate of intron gain, and is seen in the deep, ancient branches of the tree of life. This mode indicates that explosions of intron gain happened at key points in eukaryote evolution, such as the origin of animals. These different modes showcase the fact that it is difficult to determine the main theory that describes how introns arose in evolution, because there are many possible explanations for the current intron distribution.

Genomic data show that there have been approximately twice as many intron losses as intron gains in the past 1.5 billion years of eukaryote evolution (Carmel 2007b). However, because the specific lineages differ widely in the rates of loss and gain, it may be that different genes have significantly different evolution rates for intron gain and loss.

While it may never be known when introns arose, further investigation of intron position and genome evolution will

help to pinpoint better the modes of intron evolution. Many factors may contribute to both the intron's presence and role. Previous work has shown that numerous introns have their genomic position conserved between different taxa, including distantly related taxa such as animals and plants (Carmel 2007a). This would seem to imply that introns occurred very early in evolutionary history. However, there is another possible explanation for this occurrence. These conserved intron positions may occur because of proto-splice sites, which are constrained nucleotide sequences where introns are preferentially inserted (Logsdon 2004). Therefore, these conserved introns may not all have arisen early, but may have been gained later in evolution because of the preference for introns to be accumulated at these sites.

Previous work in this laboratory has catalogued intron position and phase in ribosomal protein S14 (rpS14) for multiple organisms (Nicks 2007). This gene was chosen because it has an important role in ribosome function, and because it is highly conserved in eukaryotes and prokaryotes. Since rpS14 has an essential role in the ribosome for all living organisms, it must be an ancient gene dating back to the earliest origins of life. The organisms are chosen to represent a wide range of the eukaryotic lineages and to represent all of the major branches of the eukaryotic tree of life. The expanding number of sequenced higher eukaryote genomes provides researchers with an opportunity to add additional organisms and to examine additional genes.

To investigate further the introns early theory vs. the introns late theory, several different genes were selected for analysis of intron position and phase from a wide range of eukaryotes. The eukaryotes were chosen to represent all major branches of the tree of life. The first stage of this project extended previous work on rpS17 (Nicks 2007). This protein was chosen because it is a ribosomal protein that is less conserved than rpS14. Whereas rpS14 is a highly conserved and functional component of prokaryote and eukaryote ribosomes, rpS17 has no recognizable homolog in the prokaryote ribosome. Therefore, rpS17 appears to have been added to the ribosome after divergence of eukaryotes and prokaryotes. If rpS17 is less conserved and "newer" than rpS14, it would represent a younger gene and thus might show a different pattern of intron conservation.

Based on initial comparisons of conserved introns in rpS14 and rpS17, we surveyed a few other highly conserved genes for presence of conserved introns in select taxa. Genes examined were ribosomal protein L8 (rpL8), elongation factor 2B (EF2B), and triose phosphate isomerase (TPI). We examined rpL8 because it is conserved in eukaryotes and prokaryotes, but the protein is a component of the large subunit of the protein as opposed to the small subunit. EF2B was included because it is a highly conserved gene that is used during transcription. TPI is a highly conserved and essential component of glycolysis in eukaryotes and prokaryotes. Thus, EF2B and TPI are essential, highly conserved genes that are

not components of the ribosome, and therefore might provide a different perspective on intron evolution.

Analysis of intron position conservation from these diverse genes should allow inferences about the history of introns in these genes and how the evolutionary signal differs among them. Overlaying the results of intron conservation on the eukaryotic tree of life identifies patterns where intron placement corresponds with the phylogenetic relationships. Highly conserved vs. variable intron positions provide information about the role of introns in eukaryote evolution, and contribute significant information to the debate over introns early or introns late.

## Materials and Methods

Online genome browsers were used to obtain the protein sequences by using BLAST searches. Where possible, genome browsers were also used to locate intron positions and identify each intron phase. Table 1 provides the specific websites used for each eukaryote. Some of the genome browsers did not provide complete gene structure information or the encoded protein. If this was the case, the sequence was analyzed further using EditSeq and SeqMan software in DNASTar (ver 6.0). EditSeq was used to manipulate and annotate the DNA and protein sequences. SeqMan was used to translate the

Table 1. Eukaryote Organism code and Source of DNA Sequences

Code	Species	Name	Source
Aan	<i>Aureococcus anophagefferens</i>	brown algae	http://genome.jgi-psf.org [No Introns]
Ath	<i>Arabidopsis thaliana</i>	plant	http://www.arabidopsis.org
Btl	<i>Branchiostoma floridae</i>	lancelet	http://genome.jgi-psf.org
Cel	<i>Caenorhabditis elegans</i>	worm	http://www.sanger.ac.uk
Csp	<i>Capitella species 1</i>	worm	http://genome.jgi-psf.org
Cin	<i>Ciona intestinalis</i>	Sea Squirt	http://genome.jgi-psf.org
Cre	<i>Chlamydomonas reinhardtii</i>	algae	http://genome.jgi-psf.org
Ddi	<i>Dicystidium discoidium</i>	slime mold	http://genome.jgi-psf.org
Dme	<i>Drosophila melanogaster</i>	fruitfly	http://dictybase.org/
	rpS14A and rpS14B genes		http://genome.ucsc.edu
Dpu	<i>Daphnia pulex</i>	waterflea	http://genome.jgi-psf.org
Fru	<i>Fugu rubripes</i>	puffer fish	http://genome.jgi-psf.org
Hma	<i>Hydra magnipapillata</i>	hydra	http://hydrazome.metazome.net
Hro	<i>Helobdella robusta</i>	leech	http://genome.jgi-psf.org
Lgi	<i>Lotia gigantea</i>	snail	http://genome.jgi-psf.org
Mam	human and Chinese hamster	mammal	http://genome.ucsc.edu
Mbr	<i>Monosiga brevicollis</i>	chozo flagellate	http://genome.jgi-psf.org
Ner	<i>Neurospora crassa</i>	fungus	[Tyler, 1990 #311]
Ngr	<i>Naegleria gruberi</i>	amoeba flagellate	http://genome.jgi-psf.org [No Introns]
Nve	<i>Nematostella vectensis</i>	anemone	http://genome.jgi-psf.org
Olu	<i>Ostreococcus lucimarinus</i>	green algae	http://genome.jgi-psf.org
Pbl	<i>Phycomyces blakesleeana</i>	zygomycete	http://genome.jgi-psf.org
Pch	<i>Phanerochaete chrysosporium</i>	basidiomycete	http://genome.jgi-psf.org
Ppa	<i>Physcomitrella patens</i>	moss	http://genome.jgi-psf.org
Pti	<i>Populus trichocarpa</i>	poplar tree	http://genome.jgi-psf.org
Ptr	<i>Phaeodactylum tricornutum</i>	diatom	http://genome.jgi-psf.org
Sea	<i>Stomoxys calcitrans</i>	stable fly	Genbank Accession AF119387
Smo	<i>Selaginella moellendorffii</i>	primitive plant	http://genome.jgi-psf.org
Spu	<i>Strongylocentrotus purpuratus</i>	sea urchin	http://www.ncbi.nlm.nih.gov and
Tad	<i>Trichoplax adhaerens</i>	placozoa	http://genome.jgi-psf.org
Tca	<i>Tribolium castaneum</i>	flour beetle	http://www.beechbase.org
Tps	<i>Thalassiosira pseudonana</i>	diatom	http://genome.jgi-psf.org (missing 5' exon)
Tth	<i>Tetrahymena thermophila</i>	ciliate	http://www.ciliate.org
Vca	<i>Volvox carterii</i>	chlorophyte algae	http://genome.jgi-psf.org
Xla	<i>Xenopus laevis</i>	frog	http://genome.ucsc.edu
Xtr	<i>Xenopus tropicalis</i>	frog	http://genome.ucsc.edu

genomic sequence in all three reading frames, view the aligned translations and determine specific intron boundaries.

After all of the intron positions and phases were located for all of the eukaryotes, the program MegAlign was used to align protein sequences utilizing the Clustal W method. Placement and conservation of intron position was correlated with an evolutionary tree based on currently accepted models for the tree for eukaryotes using an accepted evolutionary placement of the eukaryotes (Spiegel and Silberman, personal communication).

## Results

For each analyzed gene, a visual alignment of all protein sequences for each gene was assembled. Each protein sequence was annotated for all introns that interrupted coding sequences and noted their phase. From the alignment, conserved introns found in at least two different organisms were identified. Conserved introns were defined as those that were present in the same phase for the homologous amino acid of the protein in more than one organism. These conserved introns were then analyzed further with respect to a currently accepted evolutionary tree for eukaryotes.

Analysis of rpS14 revealed there are nine conserved introns among the eukaryotes investigated. The rpS14 sequences and intron positions are presented in Figure S1. Five of the conserved introns are in phase 0, one is in phase 1, and two are in phase 2. There are several other nonconserved introns from all phases present in this gene. Based on analysis of conserved introns in our eukaryotic tree, intron f was present only in insects. Introns b, d, and h were only found in animal lineages.

In rpS17, seven conserved introns were identified among the thirty eukaryotes studied (Figure S2). For these conserved introns, six are phase 0 and one is phase 2. Based on examination of the conserved intron table, it is clear that introns l and n are only in plant lineages, while introns k, m, and p are found exclusively in animal lineages. Intron o is only found in fungal lineages.

From the analysis of rpS14 and rpS17 it was difficult to discern any consistent pattern. The evolutionary signals from rpS14 and from rpS17 appear to be quite different. Whereas rpS14 has a mixture of phases in its conserved introns, rpS17 has almost exclusively phase 0 conserved introns. It did not appear that many introns were present in all the branches of the eukaryotic tree, which would suggest an introns recent pattern. To further investigate intron patterns, three additional widely conserved eukaryotic genes were surveyed. The genes selected were rpL8, EF2, and TPI. Ribosomal protein rpL8 is conserved between eukaryotes and prokaryotes, but is a component of the large subunit. EF2 is an elongation factor that has been used in other evolutionary studies. TPI has also been used in evolutionary studies and is an essential component of glycolysis in prokaryotes and eukaryotes. Intron patterns were examined for each of these genes from Homo sapiens, Caenorhabditis elegans, and Drosophila melanogaster. These organisms were chosen because they represent critical branches of the eukaryotic tree of life. The pattern of introns in EF2 (Figure S3) only revealed one conserved intron for the three organisms investigated while rpL8 (Figure S4) showed two conserved introns. TPI also had two conserved introns within these three organisms. TPI was chosen for further investigation because, unlike rpL8, it is not a ribosomal protein and therefore could present a different perspective on intron patterns. Intron data for TPI were then assembled from the other organisms from the evolutionary tree.

Investigation of TPI revealed a total of nine conserved introns (Figure S5). Five of the conserved introns were phase 0, three were phase 1, and one was phase 2. Introns q and y are found in plants. The conserved intron found only in animal lineages is u.

Overall, there were 25 conserved introns in rpS14, rpS17, and TPI. Out of these 25 introns, 16 were in phase 0, five were in phase 1, and four were in phase 2. RpS17 favored phase 0 introns heavily, while the other two genes had a mixture of phases in their conserved introns.

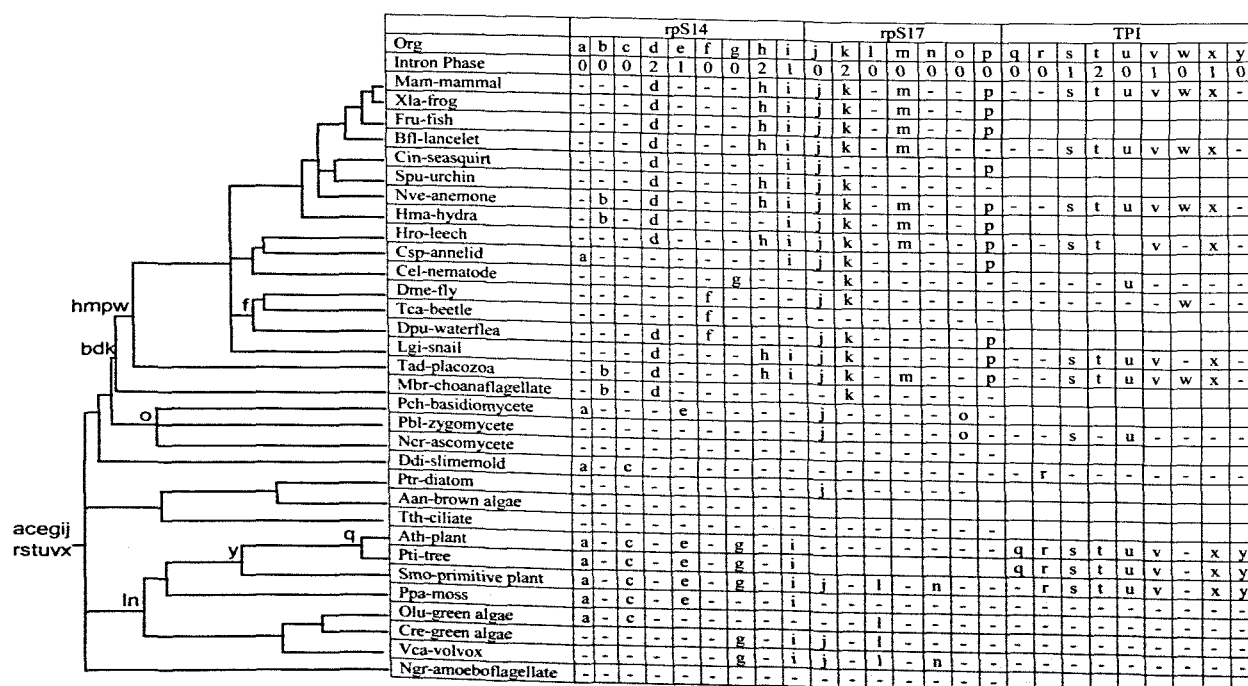
Data were used to construct a table of all the conserved introns arranged by gene, and aligned with the evolutionary tree (Figure 1). This table allows visualization of when the conserved introns arose in evolutionary history, which conserved introns were present exclusively in particular branches, and which were present in different branches. The main deep branch was that between animals and plants. In each gene there were conserved introns shared between plants and animals. In rpS14 introns a, g, and i were shared by both plant and animal lineages. Intron g was only found in nematodes and plants. Intron i was the most widely conserved intron for rpS14 among the organisms used. In rpS17 there was only one conserved intron that was shared by plants and animals – this was intron j. Out of the nine conserved TPI introns, 5 of these were shared in plants and animals. These were introns s, t, u, v, and x; all of these introns were present in approximately the same set of organisms.

## Discussion

There are a number of conclusions that can be inferred from these data about trends in intron conservation. The eukaryotic tree of life (Figure 1) was used to identify the most ancient introns to test the relevance of the introns early theory. There were twelve ancient introns in this analysis of rpS14, rpS17, and TPI. Out of these twelve introns, six were in phase 0, five were in phase 1, and one was in phase 2. The introns early theory holds that introns were present before prokaryotes diverged from eukaryotes, and that these ancient conserved introns should be in phase 0. The distribution of these ancient introns does not support the introns early theory, because there were nearly as many phase 1 introns as there were phase 0 introns. While these introns have clearly been present since the beginning of eukaryotes, this intron distribution does not support the strict definition of introns early. Another point to be made about these twelve ancient introns is that only one of these, intron j, comes from rpS17; the youngest of these three genes is present only in eukaryotes. TPI and rpS14 are present in both prokaryotes and eukaryotes, so it is logical that the older genes would contain the most ancient introns.

There were several conserved introns from each gene that were present only in animals. This may be because these introns were simply lost in other branches of the evolutionary tree. There is another possible reason for this; studies have shown that it is possible that widespread intron gain happened only during short periods of eukaryotic evolution that

**Figure 1.** Phylogenetic analysis of conserved introns for rpS14, rpS17 and TPI. Conserved introns are as indicated in the protein alignments (Supplementary Information). For each conserved intron the intron phase is indicated. Where introns likely first appeared in the tree are indicated on the branches. Three letter organism codes are based on Table 1.



coincided with major evolutionary innovations (Basu 2008). A potential example of this would be an extensive intron gain during the origin of animals (Carmel 2007b); this may be why these introns are conserved only in animal lineages. In rpS14, intron h is only present in animals. However, out of the sixteen animals sequenced, seven of them have lost this intron, which shows the genomes of different animals evolve differently.

The gene for rpS17 has several other conserved introns which appeared only in certain lineages. For example, intron l appears only in plant lineages. This is consistent with the fact that different lineages of eukaryotes lose and gain introns at rates which differ from other lineages (Carmel 2007b). The alternatives are that intron l was present early and lost very early in the branch that gave rise to animals, or that intron l was acquired very early in the branch that gave rise to plants. There are examples of conserved introns appearing only in plant lineages in all three genes investigated in this study. This fact supports the introns late theory, because it is more probable that the plant branch gained these introns than that all other branches lost it.

A peculiarity of introns c, e, g, and r was observed upon further investigation of introns that are almost exclusively in plants. These introns are all present in several plants, but occur in only one member of the animal lineage. For example, in rpS14, intron g is present in plants and in *C. elegans*, a nematode. Nematodes live in soil and therefore are frequently exposed to plants, so it is possible that this plant intron was transferred to this one specific eukaryote, and was incorporated into its rpS14 gene. Introns e and r are both present in plants and in *D. discoideum*, a slimemold. Since slimemolds are soil organisms, a similar scenario could be postulated, with the slimemold naturally acquiring a plant intron through frequent contact in its environment.

Another example of a probable intron gain event is intron f, which appears only in three arthropods: two insects and a crustacean. This intron appears to support the introns late theory, as it was gained only in this lineage within the arthropods during eukaryotic evolution. However, these three arthropods represent very old radiations estimated at  $666 \pm 58$  million years ago (Pisani 2004). Since their divergence, they have maintained these introns with no apparent changes.

Given all the data collected in this study, it is possible to conclude that introns were present in the earliest eukaryotes, and that there have been more recent intron acquisitions. The present study, therefore, suggests the introns late theory is better supported by these data. For example, intron i in rpS14 is ancient and is conserved throughout all the branches of the evolutionary tree. However, it is not present in every single eukaryote we examined, which means that it has been lost in some organisms during evolution. This type of ancient intron supports the fact that introns have been present since the beginning of eukaryotes, and subsequently have been lost by a few eukaryotes along the way. All of the ancient introns that are conserved throughout the tree of life support <https://scholarworks.uark.edu/inquiry/vol10/iss1/1>

this conclusion. A study recently concluded that all events of excessive intron gain were ancient (Carmel 2007b), so it appears that most eukaryotes acquired many of their introns early in their evolution. Then, there are the introns that are conserved only in certain lineages, such as introns q and y in TPI, which only appear in plants. These introns suggest a more recent acquisition of specific introns to specific lineages of eukaryotes. Remarkably, TPI, unlike rpS14 or rpS17, had a preponderance of introns shared in all the major lineages. For rpS14 and rpS17 there are a few introns conserved across eukaryotes, but most introns appear to be acquired in particular lineages. Conversely, TPI has 5 introns shared between plants and animals, two to three introns (q, r and y, although r may be an exception) specific to plants, and only one intron (w) specific to animals. For rpS14 and rpS17, only introns i and j are clearly conserved between plants and animals. Intron a is in a highly polymorphic region of rpS14, while introns c, e, g, are primarily in plants with single occurrences in one animal and may represent horizontal transfer rather than phylogenetic conservation.

Recent studies have expanded on the two extreme themes of introns early vs. late. Current focus is on the full spectrum of ancient, stable introns to recently gained introns (Omilian 2008). However, it is important to continue investigation of introns because their loss and gain is a slow process compared to other genetic characteristics, which allows intron positions to retain a vast amount of information about genome structure and deep evolutionary history (Irmia 2008). Analysis of single cell eukaryotes representing the base of the eukaryote tree would be helpful except that these organisms have apparently streamlined their genomes through removal of most introns. For example, the genome for the budding yeast *Saccharomyces cerevisiae* lacks introns in most genes. A survey of rpS14 genes in yeasts and fungi shows few introns and very little conservation of position (Figure S7). Therefore, we appear restricted to the higher branches of the eukaryotic tree; the multicellular eukaryotes. Further work should be pursued, continuing the analysis of genes for their intron patterns, as more data may clarify the question of the evolutionary history of gene structure.

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#### Mentor Comments:

According to mentor Douglas D. Rhoads, Maria Hester took on a challenging line of research being pursued in his laboratory and developed her own line of investigation with considerable success. He clarifies as follows:

*The work presented in Maria Hester's manuscript was the basis for her honors research in my laboratory. The pursuit of intron evolution patterns in highly conserved genes has always been a great interest to me. However, for many years the numbers of genomes available was so spartan as to not give us anything more than a few examples. With the rapid proliferation of eukaryotic genome projects representing a wide diversity of organisms, we can start to ask questions that were impossible only a few years ago. Maria's work builds upon work that I first began on rpS14. I have continued to mine new S14 genes, as they become available. Another student, Shannon Nicks started working with rpS17, which was the basis of her honors thesis. When Maria chose to pickup this project she greatly expanded the number and diversity of organisms for rpS17 and then did some great investigative work to identify TPI as an alternative. This sort of bioinformatics project has not been attractive to many students because it is so much frustrating computer work. You have to learn different genome browsers, and many of the genome sites don't readily provide the answers we need without detailed further analysis. The project is to try to learn whether the evolution of gene structure with respect to intron placement in conserved genes is a constant. There are competing theories on the timing of the origin of introns and the role of introns in gene evolution. With an ever increasing*

*diversity of genomes available, we can begin to address some of these puzzles. Maria chose to examine the evolution of gene structure in 23 widely different eukaryotic taxa using 5 different genes of varying levels of conservation. Previous data was for only 2 genes from selected taxa. Maria used genome browsers and other sequence analysis tools to deduce gene structures for ribosomal protein S17 (rpS17), triose phosphate isomerase (TPI), elongation factor 2B (eF2B), ribosomal protein L8 (rpl8), and RNA polymerase subunit 2B (RP2B). Her survey identified rpS17 and TPI as having sufficient numbers of conserved introns for detailed analysis in the entire group of organisms. Over the course of one year, she analyzed these two genes from the different available genomes, mapped introns for placement and codon phase. Her manuscript compares "intron evolution" patterns for rpS17 and TPI to my prior data for rpS14. The three genes tell very different stories, and that is the major conclusion of Maria's honors thesis. It is truly some excellent meticulous work. She has been very dedicated and has tackled some rather daunting tasks in wading through whole genome information, different genome browsers, and difficult user interfaces.*

## DEVELOPMENT OF A DISTRIBUTED ARTIFICIAL NEURAL NETWORK FOR HYDROLOGIC MODELING

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Faculty Mentor: Sreekala G. Bajwa

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### Abstract

Hydrological models are used to represent the rainfall-runoff and pollutant transport mechanisms within watersheds. Accurate representation of these dynamic and complex natural processes within a watershed is an important step in managing and protecting a watershed. Artificial neural network (ANN) models are often used in hydrologic modeling. Typical ANN models are trained to use lumped data. However, watershed characteristics used as inputs in hydrological modeling are spatially and often temporally dynamic. Therefore, a lumped model does not have the ability to represent changes in spatial dynamics of a watershed. Therefore, the purpose of this study was to develop and test a distributed ANN model for simulating the rainfall-runoff process in the L'Angeuille River Watershed located in Eastern Arkansas. The watershed was divided into nine sub-basins to account for the spatial dynamics of flow within the watershed. Inputs for the model were rainfall, average temperature, antecedent flow and curve number. Output was runoff, collected from gage-stations at Colt and Palestine representing two of the sub-basins. Daily SCS curve numbers were developed and adjusted for crop planting and harvesting dates and crop rotation practices in each sub-basin. The model had nine layers with one neuron each to represent the nine sub-basins. The layers were connected so that if one sub-basin spatially flowed into another, its output would be an input for the downstream sub-basin. The model performed well, showing  $R^2$  values of 0.93 and 0.98 and Nash-Sutcliffe Efficiency values of 0.92 and 0.97 for the validation and test datasets.

### Introduction

#### Watersheds and Watershed Management

Water is one of the most important natural resources. It "drives all human systems and those of most other organisms as well" (Heathcote, 1998). Watersheds are particularly important in managing water resources, as they are broadly defined as the area of land that contributes runoff to a particular point. Managing a watershed is crucial for maintaining good ecosystems and human health. Runoff is an important aspect of watershed management. Runoff is precipitation that falls onto the earth but does not infiltrate into the soil, evapotranspire through plants, or get stored. Runoff carries with it nutrients, sediments, and pollutants until it eventually reaches a body of water. Nutrients, sediments, and pollutants that do not get deposited along the way may end up in water

bodies. Simulation of runoff is an initial step in watershed management.

#### Hydrological Models

Hydrological modeling is a field of study that attempts to utilize mathematical and analytical models to model watersheds and predict watershed characteristics. Many hydrologic models have been developed in attempts to model different aspects of watersheds. One very common model is the Soil and Water Assessment Tool (SWAT). SWAT models are often used for modeling watersheds, but they have difficulty accounting for LULC changes other than crop rotation. This is a problem because these parameters not only vary within a watershed, but are also interrelated with one another. For example, the runoff in one section of a watershed may contribute flow into a different section of the watershed. Therefore, typical models are incapable in handling complex relationships between large amounts of data efficiently.

#### Artificial Neural Networks

Artificial Neural Networks (ANNs) were designed to process and transfer information similarly to the neurons in a human brain. Broadly, a neural network is given a variety of inputs and corresponding outputs (Figure 1). These inputs enter into a hidden layer or layers that contain neurons. As the inputs pass through the hidden layer, weights and biases are added to the data. When the weighted data goes through a neuron, it is processed with a non-linear function in an attempt to relate the input data to the target data. Simply put, ANNs have the ability to relate input and output variables in complex systems

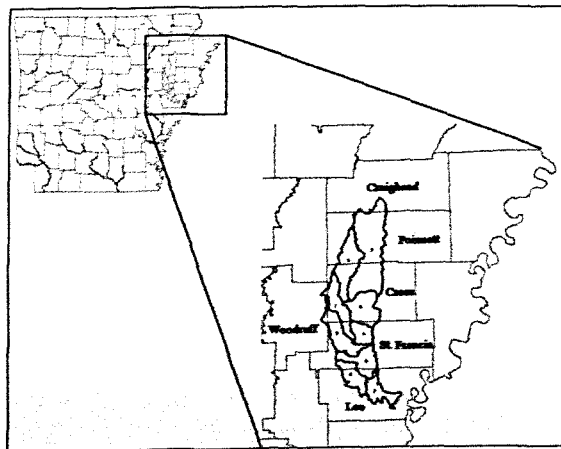


Figure 1. Location of LRW in Arkansas and counties encompassed.



(Dawson et al., 2001).

Artificial Neural Networks are relatively new to hydrologic modeling, but have the ability to handle multiple data inputs and relate them in non-linear spatial ways (Dawson et al., 2001). ANNs also have the capability to account for dynamic changes in a watershed, such as changes in land use and land cover. This property is especially important for watershed management, because increasing human population leads to a rapidly changing landscape. Typically, ANNs used in hydrologic models are feed-forward, back-propagation networks with one hidden layer of neurons. Input and target data along with network parameters are entered. Data flows forward through the network, where the network compares the computed output to the known target by calculating an error (usually mean squared error). If the error goal is not met, the network keeps re-running the data, changing the weights and biases until a given network parameter is met. The problem with this typical use of ANNs is that it does not have the ability to spatially relate the input parameters.

In this research, however, a pre-defined network in MatLab® was not used to model the LRW. Instead, a custom ANN with a specific architecture was defined in order to better capture the spatial dynamics of the flow within the watershed.

#### Significance of Research

Being able to accurately and efficiently model aspects of a watershed, particularly runoff, is very important in monitoring and controlling non-point source pollution within the watershed. Unlike point-source pollution, non-point source pollution is difficult to pinpoint and quantify. It is carried through runoff and sediment flow in and out of watersheds. Because of the Clean Water Act (1972) and its regulations, it is important to be able to quantify pollutant and sediment transport in a given watershed. Water health and quality is a good indication of ecosystem health and health of the human population. Water is the most essential resource for human survival. It is needed for drinking, for growing food, and for cleansing purposes. A lack of clean water leads to many waterborne diseases and even death. Being able to quantify, monitor, and even predict runoff and pollutant loads in runoff is a great step towards conserving and managing watersheds and water resources.

#### Objectives

In this study, an ANN model was developed to simulate and predict the watershed scale rainfall-flow process using historical flow data from USGS gage stations. Other objectives of this study were to perform a sensitivity analysis on input variables and evaluate the performance of the ANN model.

#### Methods

##### Determining Watershed for Case Study

L'Anguille River Watershed (LRW) is located in Eastern Arkansas, United States and encompasses six counties (Figure 1). LRW was divided into nine sub-basins to account for the

spatial dynamics of flow (Figure 1).

The watershed is mostly agricultural land (rice, soybean, and cotton), followed by forest and urban areas (Figure 3). LRW was chosen as a case study because, due to its large agricultural production, it has some major pollution problems.

Under section 303(d) of the Clean Water Act, states are

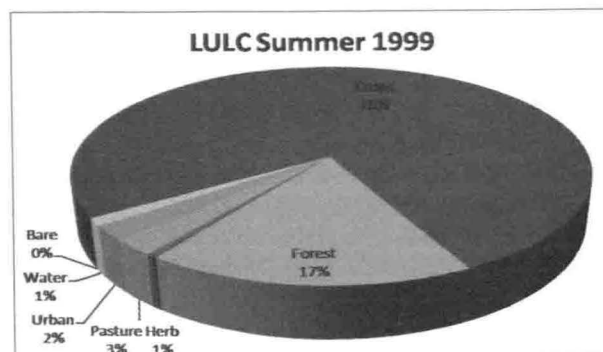


Figure 2. LULC for LRW for summer 1999.

required to develop a list of impaired waters that are too polluted or degraded to meet water quality standards set by that state (USEPA, 2009). The states are then required to establish

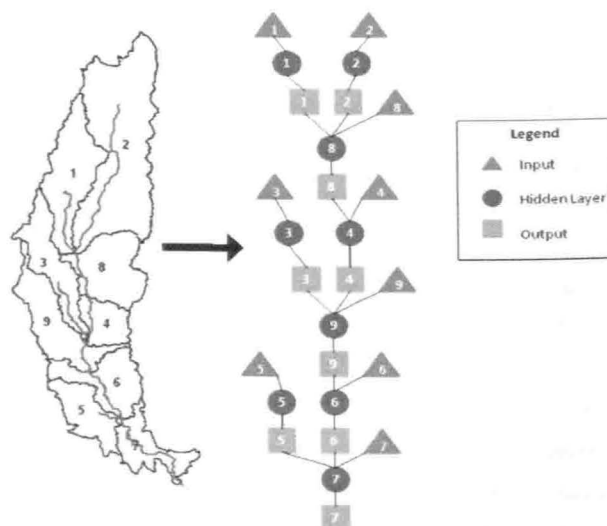


Figure 3. Simplified network architecture showing how layers were connected to account for spatial dynamics of water flow within LRW.

rankings for the impaired water bodies listed and develop Total Maximum Daily Loads (TMDLs) for the pollutant that is causing the water quality problems. Since 1995, there have been seven TMDL reports on the L'Anguille River, five for turbidity and two for fecal coliforms (USEPA, 2009). In 2008 the river had twelve of its reaches totaling over 98 miles designated as impaired (Class 5) by the Arkansas Department of Environmental Quality (ADEQ, 2008). Agriculture was the source of the pollutants and problems in all known cases (ADEQ, 2008). Five of the twelve reaches designated as



impaired in 2008 were classified as 5a streams meaning they are “truly impaired” and TMDLs need to be developed for the given parameter.

#### *Determining Input Data for ANN Model*

Runoff is precipitation that does not evapotranspire back into the atmosphere, infiltrate into the groundwater, or get stored in the soil. Therefore, to precisely quantify the amount of runoff entering a water body, it would be ideal to have exact values for these four variables. Current technology, however, does not allow for exact quantification of these parameters. Thus, there is a need to create hydrologic models that can take what data is currently available and mathematically relate the data so as to estimate runoff. Inputs chosen for this model were precipitation, average temperature, Soil Conservation Service Curve Number (SCS-CN), and antecedent stream flow where available. Precipitation and average temperature data for each sub-basin were collected from the nearest weather station; data used were from January 1, 1995–December 31, 2004. Antecedent stream flow data were collected from two different USGS gage stations on L’Anguille River at Colt and Palestine for the same years. The daily SCS-CN had to be developed based on LULC and hydrologic soil type data.

#### *Development of SCS-CN*

The SCS-CN provides a way to quantify and estimate the amount of runoff that an area of land generates, based on the LULC and hydrologic soil type of that land. Since the precipitation data were from January 1, 1995–December 31, 2004, daily SCS-CN’s needed to be developed for this time period for each sub-basin. LULC data for LRW were available for spring, summer, and fall of 1999 from the University of Arkansas’ Center for Advanced Spatial Technology database, so it was assumed to be the base LULC of the 1995 data. Soils data were available for LRW from the U.S. National Resource Conservation Service. Using ESRI’s ArcGIS program, and specifically ArcMap, the soils and LULC data were dissolved (based on hydrologic soil group and cover name, respectively) and intersected for each sub-basin. Then, the SCS-CN was calculated for each soil-LULC complex based on NEH curve number tables (USDA, 2008). The area weighted CN was then calculated for each sub-basin for the spring, summer, and fall datasets.

Next, the CNs were adjusted in order to account for crop planting and harvesting dates (USDA, 1997) because the CN changes based on whether or not the crop is actually in the ground. The result after this adjustment was a daily CN for one year, assumed to be year 1995 (the beginning of the precipitation, temperature, and gage station data). Since crop rotation is a significant management practice in agriculture, the first year’s daily CNs had to be adjusted according to common crop rotation practices. These crop rotation practices were determined based on a focus

group survey of University of Arkansas Cooperative Extension Service agents (Hill et. al, 2003). The data produced after crop rotation adjustment included 10 years (Jan. 1, 1995– Dec. 31, 2004) of daily, area-weighted CNs for each sub-basin.

#### *Determining Network Outputs and Target Data*

Since the purpose of this study was to predict the flow in the L’Anguille River, naturally the target output for the model was discharge. However, there are only two USGS gage stations along the entire reach of the L’Anguille River (at the outlets of sub-basins eight and nine). Therefore, only sub-basins eight and nine were connected to target data and used to monitor and evaluate network performance.

#### *Constructing Network Architecture*

One objective of the project was to create an ANN that could account for the spatial dynamics of flow within the watershed. Instead of using a pre-defined network in MatLab®, a custom, distributed ANN with a unique architecture was defined in order to better capture the spatial dynamics of the runoff within the watershed. By custom defining the network, the architecture was arranged in such a way that the output of one sub-basin was an input into another sub-basin if the first sub-basin’s flow entered into the second sub-basin.

The network created contained three or four inputs for each sub-basin (rainfall, average temperature, SCS-CN, and antecedent stream flow for sub-basins 8 and 9), nine layers with one neuron representing each sub-basin, and target output for sub-basins eight and nine. The network layers were

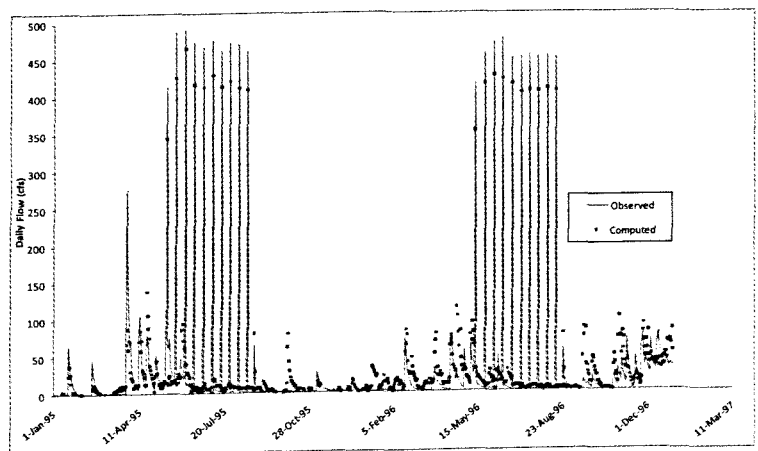


Figure 4. Hydrologic time series for validation set for sub-basin 8.

connected in such a way as to account for the spatial dynamics of water flow between the sub-basins within LRW (Figure 4).

For network training, the Levenberg-Marquardt algorithm was used as the training algorithm and the performance of the network was measured by the Mean Squared Error (MSE). The data sets were divided into training, testing, and validation datasets. Because the training of the network requires the most

data, 60% (1997-2002) of the dataset was used for training, whereas 20% (1995-1996) was used for testing and 20% (2002-2004) for validation.

### Optimization of Network Parameters

Because only one neuron was used in each layer, it was not necessary to optimize the number of neurons. Thus, optimization was performed only on the training parameters. Since the training function chosen was the Levenberg-Marquardt algorithm, the only option for optimization of the network was the learning rate. A trial and error procedure was followed by varying learning rate at different increments. The optimized learning rate was identified as the one that resulted in the lowest MSE.

### Sensitivity Analysis

The sensitivity of the model to each input was determined, by examining the weights of the network inputs. It was determined that the model was "sensitive" to all inputs of the model (rainfall, average temperature, SCS-CN, antecedent flow).

### Results

The custom defined neural network was run, using the optimized learning rate of 0.15. The simulated flow closely followed the actual flow (Figures 5 and 6). The model was evaluated at the outputs of sub-basins eight and nine using three different criteria: (1) correlation coefficients between

Table 1. Calculated results of neural network model performance.

		Correlation Coefficient	R-Square Value	Nash-Sutcliffe Efficiency
Sub-basin 8	Training	0.82	0.68	0.68
	Testing	0.74	0.55	0.46
	Validation	0.96	0.93	0.92
Sub-basin 9	Training	0.98	0.96	0.96
	Testing	0.96	0.93	0.93
	Validation	0.99	0.98	0.97

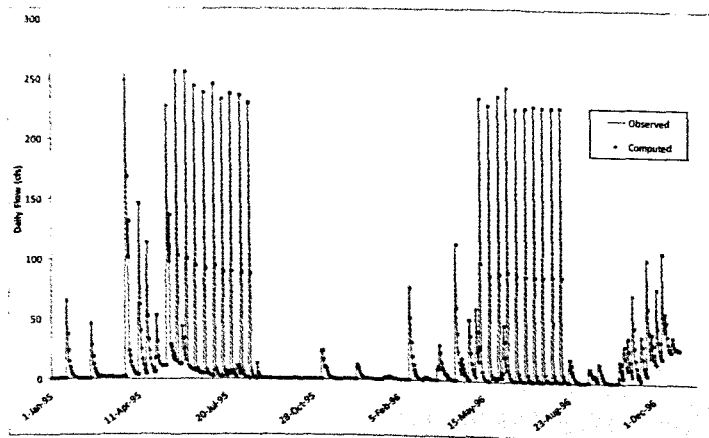


Figure 5. Hydrologic time series for validation set for sub-basin 9.

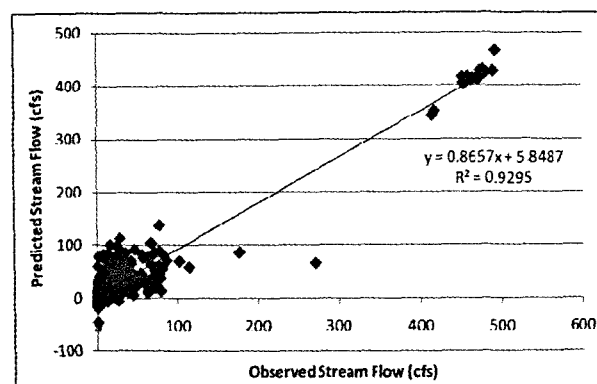


Figure 6. Linear regression of observed stream flows versus computed stream flows for sub-basin 8.

computed and observed results, (2) R-square values between the computed and observed results, and (3) Nash-Sutcliffe Efficiency coefficient.

Linear regression was performed using Excel© (Figures 7 and 8). Both the correlation coefficient and R-square value

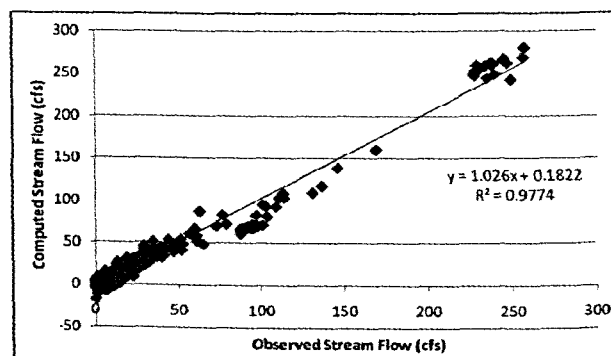


Figure 7. Linear regression of observed stream flows versus computed stream flows for sub-basin 9.

were calculated for sub-basins 8 and 9 for the training, testing, and validation datasets (Table 1).

The Nash-Sutcliffe Efficiency value is often used in evaluating hydrologic models because it is "insensitive to additive and proportional differences between model simulations and observations" (Harmel et. al, 2007). The Nash-Sutcliffe Efficiency value was calculated for sub-basins 8 and 9 for the training, testing, and validation datasets using equation 1 (Table 1).

The performance of the model was very good in sub-basin 9, but poor in sub-basin 8, particularly for training and test data. This may be because the model optimized itself for sub-basin 9 and not sub-basin 8.

### Summary and Conclusions

The goal of this project was to develop a spatially distributed, custom ANN to model the flow within a watershed. The model was trained using historical daily rainfall, average temperature, SCS-CN, and stream flow data. The model was trained with 6 years of data

and tested and validated using 2 years of data. The results of the model show that the model was able to simulate the stream flow at sub-basin 9 very well, with all R2 values >0.93 and the Nash-Sutcliffe Efficiency values all being greater than 0.93 as well. Sub-basin 8 results were not quite as good as sub-basin 9; however, the results are acceptable with training and testing R2 values >.55 and validation R2 = 0.93. Also, the Nash-Sutcliffe

$$NSE = 1 - \frac{\sum_{i=1}^n (obs_i - comp_i)^2}{\sum_{i=1}^n [(obs_i - \overline{obs})]^2}$$

Efficiency values were all positive and greater than 0.46. The better performance of the model at sub-basin 9 could be due to the fact that it is spatially further down from sub-basin 8, and therefore had more previous inputs.

Table 1. Calculated results of neural network model performance.

		Correlation Coefficient	R-Square Value	Nash-Sutcliffe Efficiency
Sub-basin 8	Training	0.82	0.68	0.68
	Testing	0.74	0.55	0.46
	Validation	0.96	0.93	0.92
Sub-basin 9	Training	0.98	0.96	0.96
	Testing	0.96	0.93	0.93
	Validation	0.99	0.98	0.97

In conclusion, this study has shown that a spatially distributed ANN is very capable at accurately simulating the stream flow of a river in a watershed. A lumped, SWAT model that was developed for LRW using total monthly flows instead of daily flows had R2 values between measured and predicted total flows of 0.84 and 0.87 for calibration and validation periods, respectively (Srivastava, et. al, 2005). The Nash-Sutcliffe Efficiency values were also 0.86 and 0.91 for calibration and validation periods, respectively. For both sub-basin 8 and sub-basin 9 validation data sets, the R2 and Nash-Sutcliffe Efficiency values were higher than the SWAT model. This shows the capabilities and possibilities that a distributed artificial neural network has in modeling stream flow.

### Acknowledgements

The authors thank the Arkansas Department of Higher Education for funding of this project through a State Undergraduate Research Fellowship. Thanks are also due to Dr. Findlay Edwards and Dr. Marty Matlock for their guidance and support, and to Mansoor Leh for his help on ANNs and ArcGIS.

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### Mentor Comments:

Professor Sreekala Bajwa reflects on the innovative quality of Rebecca Logsdon's work and the reasons why it merits publication.

*Rebecca Logsdon's research merits publication in the Inquiry journal for a number of reasons. Her work is quite innovative as there are no published articles on the distributed artificial neural network model for representing watershed scale hydrological processes in large watersheds. When Rebecca started this project, her background in ecological engineering, particularly in hydrology, geographic information systems (GIS), statistics, etc was very minimal. She taught herself many of the basic concepts, and learned the relevant information quickly. The quality of the work she has done is excellent. The fact that her research is accepted for presentation at the World Congress on Computers in Agriculture and Natural Resources is a testimony to its quality and relevance in today's world.*

*Artificial neural networks (ANN) models are usually fast, accurate and easy to implement. They have been used in hydrologic modeling for simulating rainfall-runoff, groundwater movement as well as nutrient and pollutant transport. The practical use of ANN models in hydrology was fairly limited in the past due to the lump nature of these models. Watershed scale hydrologic processes are highly distributed processes as the soil, topography, vegetation*

*and weather vary spatially and temporally in a watershed. Therefore, Rebecca focused on developing a watershed scale distributed ANN model to represent the spatial and temporal dynamics of rainfall runoff. Such a model has the potential to broaden its application to flood forecasting, water quality modeling, water planning, understanding the impact of urban development, etc. Therefore, her research is an important step towards utilizing innovative modeling tools that are faster and easier to run towards protecting the environment.*

*Rebecca's work is high quality and fairly complex for an undergraduate research project. She devoted an enormous amount of time to complete this work. This work has served as a platform for her success in attracting an NSF fellowship and admission to graduate school at Purdue University.*

## ASSESSMENT OF CARDIAC FUNCTION IN LCAD DEFICIENT MICE AFTER A SINGLE BOUT OF ENDURANCE EXERCISE

By Evan M. Lord

Department of Health Science, Kinesiology, Recreation and Dance

Faculty Mentor: Charles E. Riggs

Department of Health Science, Kinesiology, Recreation and Dance

### Abstract

*Fatty acids are the primary fuel source for cardiac tissue in both humans and animals. These become especially important during times of starvation and long bouts of exercise. Fatty acids are broken down into smaller, useable acyl-CoA subunits through a process called beta-oxidation. The first step in this process must be catalyzed by one of four acyl-CoA dehydrogenase enzymes depending on the length of the fatty acid to be metabolized. The enzyme that catalyzes the breakdown of long-chain fatty acids, long-chain acyl-CoA dehydrogenase (LCAD), was examined. The objective of this study was to determine how a single bout of endurance exercise impacts cardiac function in LCAD deficient mice as well as to assess whether recovery from exercise is adversely affected. Mice were separated into four groups for testing: LCAD, non-exercised; LCAD, exercised; control, non-exercised; and control, exercised. Mice were exercised by forced running at a speed of 31 m/min with increasing grade (2%) each 20 min. Twenty-four hours post-exercise, the mice were anesthetized with sodium pentobarbital (40 ml/kg body weight,) and ejection fraction, fractional shortening, and cardiac output values were determined in both groups. It was determined that exercise had no effect on cardiac function or recovery in either the LCAD or control group. There was, however, a significant difference between the LCAD and control group for fractional shortening ( $p < 0.05$ ) and cardiac output ( $p < 0.05$ ). It was concluded that a single bout of endurance exercise had no significant effect on the cardiac function of LCAD deficient mice during recovery, although the disease did negatively affect cardiac function when compared to the control group.*

### Introduction

In order to perform its numerous tasks, the body must constantly produce useable energy from chemical compounds such as carbohydrates, proteins, and fats. Fats, specifically fatty acids, are stored as triglycerides in both adipose tissue and skeletal muscle until they are needed for energy production. The energy stored in these compounds is released through a process known as beta-oxidation (Schulz, 1991). Because the body depends on numerous sources of fuel, including fats, to supply it with energy, beta-oxidation plays a key role in the regulation and maintenance of metabolic homeostasis in the body. Fatty acid degradation in the mitochondria is an essential process for energy production during periods of fasting and other metabolic stresses.

Fatty acids broken down through beta-oxidation produce adenosine triphosphate (ATP) that is used by the body to maintain physiologic function, particularly in cardiac tissue and skeletal muscle. ATP produced via oxidation of fatty acids is primarily depleted by these tissues during times of prolonged exercise and also plays a major role in supplying energy while the body is at rest (Coyle, Jeukendrup, Wagenmakers and Saris 1997). The ATP produced from fatty acids via beta-oxidation, the Krebs cycle, and electron transport may also fuel the body in times of fasting in order to help regulate the body's blood glucose levels since glucose, a molecule that can be oxidized to produce ATP, is no longer being supplied by the diet (Schulz, 1991).

Within the last thirty years, people have begun to be diagnosed with deficiencies of ACDs and other enzymes that affect fatty acid metabolism (Ventura, Ruiter, Ijlst, Tacaes de Almeida, and Wanders, 1998). Although cases of fatty acid oxidation disorders (FODs) are rare when compared with other metabolic diseases, the effects of these disorders can be very severe and are often fatal in younger patients. FODs are now known to be among the most common of genetically inherited metabolic disorders and frequently present the individual with life-threatening conditions. While more information is being uncovered about FODs, there is still much that is unknown about them due to their rare occurrence in humans.

The presence of an LCAD deficiency in mice produces symptoms that are most closely associated with those seen in humans with a VLCAD deficiency (Kurtz et al. 1998a). Characteristics of LCAD deficiency in mice may include sudden death, fasting and cold intolerance, hypoketotic-hypoglycemia, and an increase in the fatty content of the liver and heart. Furthermore, LCAD deficiency may be a cause of skeletal myopathy in matured mice as well. Exercise has been hypothesized to play a part in the causation or stimulation of all the symptoms mentioned above (Strauss et al., 1995; Mathur et al., 1999). LCAD deficient mice also experience excessive gestational loss of both LCAD  $-/-$  (40%) and LCAD  $+/-$  (50%) pups. While much is known about LCAD deficiency in humans and mice, there are relatively few published studies.

While use of mice with LCAD deficiency is common, a human case of the deficiency has yet to be found. This may be due to an increased risk of gestational loss as seen in the mouse model (Schuler & Wood et al. 2002). One of the most common FAO disorders found in humans is that associated with very-

long chain fatty acids (VLCAD). Symptoms associated with human VLCAD deficiency during infancy include nonketotic hypoglycemic coma, cardiac hypertrophy, endocardial fibroelastosis, hepatomegaly, Reye-like syndrome, sudden infant death syndrome (SIDS), and lipid storage myopathy (Hale & Bennett, 1992). While VLCAD deficiencies are the most prominent and easily diagnosed type seen in humans, onset of the disorder during adolescence or early adulthood is still very difficult to detect. Mouse models of FOD have introduced the opportunity to investigate the physiological effects of these deficiencies in humans and may potentially lead to earlier and easier detection. LCAD deficient mice have become a valuable model in the study of triglyceride deposition in both the human and animal heart.

When the oxidation of long-chain fatty acids is impaired, both cardiac and muscle tissues can be severely affected (Ogilvie et al., 1994). There are five studies suggesting that exercise may amplify VLCAD deficiency in humans (Ogilvie et al., 1994; Strausberg et al., 1997; Smelt et al., 1998; Minetti et al., 1998; Merinero et al., 1999). Importantly, the patients in the above studies represented a variety of ages and all complained of abnormal muscle pain and weakness during exercise. Further, evidence from previous experiments indicates that VLCAD deficiency in mice may cause a delay in the recovery of skeletal muscle from exercise. It is unclear whether the delay is associated with a reduced ability to resynthesize ATP and phosphocreatine (energy sources), or the reduced ability to resynthesize muscle glycogen (carbohydrate) stores. It is also unknown whether or not the delayed recovery is associated with either rhabdomyolysis or prolonged fatigue. On the whole, little is known about how exercise affects skeletal muscle and cardiac function in animals with LCAD deficiency.

Another area of interest is whether FOD disorders specifically have an effect on the ejection fraction and fractional shortening capabilities of the heart. The ejection fraction measures the percentage of volume of blood that is ejected with each ventricular contraction. Ejection fraction is calculated using a ratio that compares the volumes of the left ventricle during both systole and diastole ( $LV_{diastole} - LV_{systole} / LV_{diastole}$ ).

Fractional shortening is another measurement that can be used to assess left ventricular performance. Like the ejection fraction, fractional shortening measures the efficiency of the left ventricle. Instead of using volumes, fractional shortening is based on the change in the diameter of the left ventricle during systole and diastole ( $LV_{diastole} - LV_{systole} / LV_{diastole}$ ). This method focuses less on volume change and more on cardiac tissue function during both systole and diastole. A heart that is not functioning properly, therefore, would be expected to produce a lower fractional shortening percentage than a normally functioning heart. In the mouse model, the average value for fractional shortening is approximately 57% with values below 40% being indicative of cardiac malfunction or improper left ventricle performance (Gardin et al. 1995). When extrapolated,

the results produced by the mouse model provide a positive correlation with the average known values of the human model.

It was anticipated that the values obtained for ejection fraction, fractional shortening, and cardiac output of LCAD deficient mice would be lower than those seen in wild type controls both during rest and after periods of endurance exercise due to their inability to properly use long-chain fatty acids to provide energy to the heart during these times.

## Objective

The objective of this research was to determine if recovery from endurance exercise is delayed in LCAD deficient mice and if cardiac function is adversely affected. The effects of LCAD deficiency and acute exercise on cardiac function were assessed by examining specific cardiac dimensions, the ejection fraction, fractional shortening, and cardiac output.

## Materials and Methods

Procedures used in this study were based on the protocol reported by Johnson and Riggs (2002). Cardiac function was assessed via echocardiography in normal (129 SvJ.C57BL6) mice ( $n=10$ ) and mice (129 SvC57BL6 knockout) homozygous for an LCAD deficiency ( $n=10$ ). All procedures were identical for each group of enzyme deficient mice (pre- and post-exercise) as well as each control group (pre- and post-exercise). Mice were divided into four groups: 1) non-deficient, non-exercised; 2) enzyme deficient, non-exercised; 3) non-deficient, exercised; 4) enzyme deficient, exercised. Mice were exercised by forced running at 31 m/min with increasing grade (2%) each 20 min. Exercise was terminated upon exhaustion. Twenty-four hours post-exercise the mice were anesthetized and cardiac function was determined via echocardiographic methods. In vivo testing of the contractile properties of the heart was conducted using echocardiography after anesthetizing the mouse using sodium pentobarbital (40 mg/kg body weight) administered interperitoneally. Data were collected before and after exercise in LCAD and control mice and the results were compared both within groups and between groups. The echocardiographic methods used to evaluate cardiac function provided results consistent to those previously reported by Rottman et al. (2003). Stroke volume was determined by tracing the outline of the left ventricle during systole and diastole. Measurements were taken for both short- and long-axis views. Heart rate was determined by placing needle electrodes in the right and left shoulder of the mouse as well as a ground wire in the leg. The highest registered reading was used in the computation of cardiac output. Cardiac output was determined by multiplying the value obtained for stroke volume by the value obtained for heart rate ( $CO = SV \times HR$ ).

## Statistical Analysis

Data were analyzed utilizing analysis of variance (ANOVA) procedures in a 2 x 2 factorial design. The factors were enzyme deficiency (LCAD deficient, Control) and exercise (Exercised, Non-exercised). All comparisons were made at the  $p < 0.05$  level of significance.

## Results

Complete data were collected for  $n = 10$  mice in both the LCAD and control groups for all variables (EF, FS, SV, HR, CO).

Table 1:

Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.864	0.098	0.750	1.000
Fractional Shortening	FS%		61	1.79	58.1	63.6
Stroke Volume	SV	mL	0.05	0.00483	0.04	0.05
Heart Rate	HR	bpm	370.4	22.85	340	410
Cardiac Output	CO	mL/min	17.32	1.146	15.08	18.90

Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.855	0.104	0.750	1.000
Fractional Shortening	FS%		60.9	2.91	57.1	65.6
Stroke Volume	SV	mL	0.05	0.00483	0.04	0.05
Heart Rate	HR	bpm	369.3	27.06	334	415
Cardiac Output	CO	mL/min	17.24	0.860	16.00	18.35

Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.870	0.148	0.750	1.000
Fractional Shortening	FS%		65.8	2.21	62.2	69.0
Stroke Volume	SV	mL	0.05	0.00483	0.05	0.06
Heart Rate	HR	bpm	361.0	29.13	315	408
Cardiac Output	CO	mL/min	19.04	1.267	17.25	21.36

Category	Name	Units	Mean	SD	Minimum	Maximum
Ejection Fraction	EF		0.888	0.099	0.750	1.000
Fractional Shortening	FS%		64.3	2.52	61.2	68.6
Stroke Volume	SV	mL	0.05	0.00421	0.05	0.06
Heart Rate	HR	bpm	369.3	27.06	310	405
Cardiac Output	CO	mL/min	18.95	0.889	17.50	20.25

### Ejection Fraction

Ejection fraction values were determined before and after exercise for both experimental groups. The average value of ejection fraction for the LCAD group before exercise was found to be  $0.864 \pm 0.098$  and the average EF after exercise in the same population was  $0.855 \pm 0.104$ . Average values of ejection fraction were also obtained for the control group before and after exercise. These values were found to be  $0.870 \pm 0.148$  and  $0.888 \pm 0.099$ , respectively.

There was no significant difference between the values obtained for either group as a result of exercise. There was also no significant difference between the LCAD and control groups.

### Fractional Shortening

Values for fractional shortening were obtained before and after exercise for both experimental groups. Averages were determined for all groups and are included in Table 1. The average value of FS for the LCAD group before exercise was  $61 \pm 1.79$  and the average value after exercise was  $60.9 \pm 2.91$ . Data were also compiled and averaged for the control group which produced results of  $65.8 \pm 2.21$  (%) and  $64.3 \pm 2.52$  (%) before and after exercise respectively.

There was no significant difference in fractional shortening as a result of exercise. There was, however, a significant difference present between the average values obtained for the LCAD and control groups ( $p < 0.05$ ). A graphical interpretation of the results can be seen in Figure 1 below.

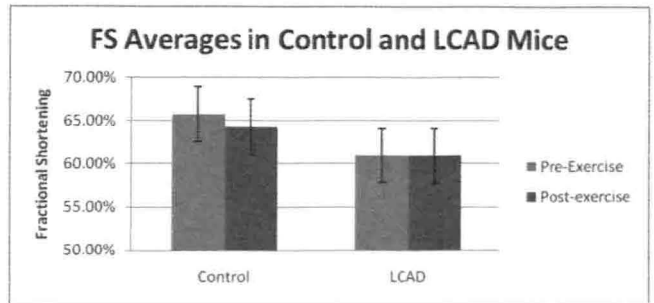


Figure 1: A significant difference was present between the control and LCAD groups both pre- ( $p = 0.001$ ) and post-exercise ( $p = 0.013$ ).

### Cardiac Output

Cardiac output values were determined for both LCAD and control groups before and after exercise. The average values for cardiac output for the LCAD group were found to be  $17.32 \pm 1.146$  (mL/min) before exercise and  $17.24 \pm 0.860$  (mL/min) after exercise. The control group produced averages of  $19.04 \pm 1.267$  and  $18.95 \pm 0.889$  for the pre- and post-exercise groups, respectively. While no significant difference was found as a result of exercise, there was a significant difference between the LCAD and control groups ( $p < 0.05$ ). Figure 2 shows the mean values of cardiac output of both the LCAD and control mice before and after exercise.

### Discussion

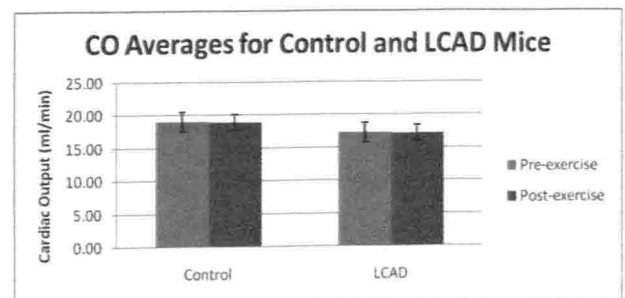


Figure 2: A significant difference was present between the control and LCAD groups both pre- ( $p = 0.005$ ) and post-exercise ( $p = 0.001$ ).

Variation of data was seen within individuals in both groups and across all variables. Discrepancy in the data produced for each individual can largely be attributed to the natural variation in homeostatic conditions commonly seen in both humans and animals. It is also possible that the condition of the mouse (stress level/activity level) at the time of testing had an impact on cardiac function. However, it is likely that any such effect was minimal. In order to produce a data set more indicative of the test group as a whole, averages were calculated and used for statistical analysis.

The values for ejection fraction for LCAD deficient mice were expected to be below normal during rest and after periods of endurance exercise when compared to the control group. Statistical analysis of the data showed that there was no significant difference in the values obtained within either group

as a result of exercise. It was also determined that there was no significant difference between the LCAD mice when compared to the control group. Ejection fraction aims to measure the volume change seen in the left ventricle during each contraction of the heart. As previously stated, this is done by comparing the volume of the left ventricle during diastole to the volume of the left ventricle during systole. The data provide relevant information on the cardiac function of LCAD mice. It was concluded that a single bout of endurance exercise has no effect on ejection fraction in LCAD deficient mice. It can be deduced that the inability of these mice to use long-chain fatty acids during endurance exercise, a time when fatty acids are the primary fuel source, poses no detrimental effects to cardiac function during recovery.

Data were also collected for fractional shortening in both LCAD and control groups. The means for both experimental groups before and after exercise compared favorably to the expected values presented in studies by both Gardin et al. (1995) and Rottman et al. (2003). Because there was no significant difference between the values obtained for each experimental group as a result of exercise, it can be concluded that LCAD enzyme deficiency has no effect on cardiac function during recovery from exercise in LCAD deficient mice. There was a significant difference, however, in the fractional shortening values of the LCAD group when compared to the control group both before and after exercise. This finding is of particular interest, especially because there was no significant difference in the ejection fraction values obtained from the two groups. The disparity present in the data could possibly point to some type of dysfunction in the cardiovascular system of LCAD deficient mice. A more in-depth examination of fractional shortening in the LCAD mouse population would be necessary to develop further conclusions regarding the reason for the decreased fractional shortening values.

Analysis of the cardiac output values collected from LCAD deficient mice before and after exercise showed that endurance exercise had no significant effect on cardiac output during recovery. This indicates that the inability of LCAD mice to effectively metabolize fatty acids for use in cardiac muscle tissue during endurance exercise had no effect on overall cardiac function after recovery. However, statistical analyses did reveal that cardiac output values obtained for the LCAD group were significantly lower than those of the control group. It is possible that this discrepancy can be explained by the fact that, in general, control mice are heavier than LCAD mice. Because this is true, control mice generally have bigger hearts and would, therefore, be expected to have greater overall cardiac output. Since heart size was not taken into account when calculating cardiac output, a one-way ANOVA was run analyzing the weights of both experimental groups; control mice were indeed significantly larger than LCAD mice ( $p < 0.05$ ). It can be reasonably assumed that, because the control mice are larger, they have larger hearts. This would adequately explain the difference seen in cardiac output measurements between the LCAD and control mouse experimental groups. It cannot be concluded,

however, whether this was the true cause for discrepancy as heart weight was not taken for the two experimental groups.

The data collected for both the LCAD and control groups were similar to those of Rottman et al. (2003). This not only supports previous research on cardiac function in mice, it also further promotes the efficacy of echocardiography as an effective assessment technique for measuring cardiac output in mice. The determination of cardiac output via echocardiographic methods is less intrusive than many of the other practices currently employed in the evaluation of cardiac function in mice.

This research will contribute to the base of research supporting echocardiography, although more is still needed in order for it to become a common tool for analysis of cardiac function in mice. While answers to specific questions regarding cardiac function in LCAD deficient mice have been addressed through this research, it has also raised some additional questions. Research should address whether the cardiac output would be affected if the mice were put through a training regimen rather than a single session of endurance exercise. Research should also examine whether the age of an LCAD deficient mouse plays a role in cardiac function and whether cardiac function of LCAD deficient vs. normal mice would differ if readings were taken directly after exercise. Further, role of heart size in identified differences in CO data between LCAD and control mice could be investigated, along with determination of other physical or physiological reasons that LCAD mice appear to have decreased cardiac output when compared to control mice.

## Conclusion

After extensively testing the contractile properties of the mouse heart via echocardiography, it was concluded that LCAD enzyme deficiency has no serious detrimental effects on cardiac function during recovery from exercise in LCAD deficient mice. It was also determined, however, that LCAD enzyme deficiency negatively affects cardiac function in LCAD deficient mice when compared to wild type control mice. LCAD deficient mice consistently displayed lower averages for both fractional shortening and cardiac output than control mice, which is indicative of decreased overall cardiac function.

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#### Mentor Comments:

In his mentor letter, Professor Charles Riggs points out that Evan Lord's work has implications for understanding how fatty oxidation disorders affect cardiac function.

*The work completed by Evan Lord is an extension of work done by previous students with my guidance. He received an Honors College Undergraduate Research grant in the Fall 2008 to begin work on his project. He completed the project in the Spring 2009.*

*His work concentrated on the effects of fatty acid oxidation disorders and exercise on cardiac function using transthoracic echocardiography. While the technique has been used to evaluate the effects of a variety of conditions on the structure of the heart it has been used sparingly to evaluate cardiac function. Since one of the consequences of fatty acid oxidation disorders is frequently a decline in cardiac function, Mr. Lord decided to determine if echocardiography could be used to determine cardiac function in these mice and to determine if the disorder and the combination of the disorder and exercise would have an effect on function of the heart.*

*It is clear that fatty oxidation disorders can have significant consequences for people of all ages. Little research has been done examining the effects of exercise on mice with these disorders so his work will make valuable contributions to knowledge about the disease and also about the capabilities of affected animals to exercise.*

## FRAMING THE FOREIGN FEMININE: PORTRAYALS OF MIDDLE EASTERN WOMEN IN AMERICAN TELEVISION NEWS

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### Abstract

*This study examines whether American television news coverage uses negative agenda-setting to depict Middle Eastern women. In approaching coverage of the Middle East, one of the key issues has been the plight of Middle Eastern women in their Islamic societies. Qualitative scholarship and limited quantitative analyses of print portrayals argue that Western media depict Muslim/Middle Eastern women negatively. However, there appears to be no research documenting how American television news, neither network nor cable, portrayed these women.*

*I conducted a content analysis of 61 news packages from ABC, CBS, and NBC along with Fox News and CNN, aired between September 11, 2001, and December 31, 2008, to answer the questions I had concerning how television news portrayed these women. I was curious to see if television journalists fell into the habit of depicting Middle Eastern women as submissive, second-class citizens, which was the accusation leveled at the Western press in prior scholarship. In addition to a quantitative analysis to determine the actual elements of coverage (frequency of portrayals and prevalence of frames), a qualitative analysis of anchor lead-ins was also conducted. This study revealed that Middle Eastern/Muslim women were depicted more negatively than positively. They were often portrayed as submissive victims of their societies, an "othered" entity requiring rescue through Western intervention. Palestinian and Iraqi female suicide bombers were also portrayed as threats while being depicted simultaneously as victims. In short, this initial research analyzing American television media discovered networks and cable channels do practice negative agenda-setting in regard to portrayals of Muslim women and their societies. The following article is a synopsis of a longer work exploring the extensive literature on women in the Middle East.*

### Introduction

This study examines whether American television news coverage adopts a negative news slant regarding Middle Eastern women. Wilkins (1995) found "given most of what we know about other regions is received through mass-mediated sources, images transmitted through the news play a critical role in the shaping of knowledge about perceptions of persons from other cultures" (p. 50). Wilkins goes on to report, "Eastern women have most often been described in Western literature and popular media as subservient figures, suffering from ethnic or religious oppression" (p. 51).

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While past research has been well documented regarding agenda-setting, frame-setting, and the news coverage of the Iraq War (Kull, Ramsay & Lewis, 2002), research directed toward portrayals of Middle Eastern women has focused solely on the print media. In fact, much of the past research used in this study to formulate an approach to studying news portrayals of Middle Eastern and Islamic women in broadcast media has been drawn from print and other research findings. Sharia Fahmy (2004) analyzed Associated Press wire photographs of Afghan women before and after the Taliban regime, and her research is the first dealing directly with media portrayals of Middle Eastern women. She found that AP photographs largely depicted women in negative frames, leading me to question whether television news might also portray Middle Eastern women negatively. Fahmy's research has been widely cited, illustrating the need to examine the issue further by incorporating a look at broadcast news depictions.

Research in this field is necessary to evaluate perceptions Americans may develop from interpreting news coverage regarding the Middle East. It is important to discover whether the media may be propagating a negative stereotype of oppression of Middle Eastern women that could influence Americans' perceptions. If American television coverage depicts Islamic societies through a negative lens, the American public could likewise develop a negative, and perhaps inaccurate, perception of Islamic culture.

Due to the lack of prior research establishing the nature of television news coverage regarding Middle Eastern women, the present study analyzes American television news as a whole. The purpose is to describe the composition of coverage and to identify portrayal practices across networks. Examination of network and cable channel differences in news coverage and possible biases would require a far broader sample than the 61 stories that comprised this sample, although such an examination would undoubtedly be valuable. In addition, this article is an abbreviated version of a much longer work that incorporated literature from a wide variety of academic fields and also discussed Orientalism, veiling practices and uses, and Arab feminism. However, in the interest of space, only the literature needed to explain the methods carried out in this research was included in this article.

### Literature Review

Past research on agenda-setting, frame-setting, and depictions of Middle Eastern/Muslim women provides the basis for this research. Methods, variables, and operational definitions,

are based on research from a variety of disciplines, as no work was found that examined specific portrayals of Middle Eastern women in American television news coverage.

### *Agenda-Setting and Frame-Setting Literature*

In agenda-setting theory, the first level of agenda-setting is concerned with issue salience. First level agenda-setting argues that "media transfer issue salience to audience agendas by covering some topics more than others" (Aday, 2006, p. 767). Whereas first level-agenda-setting theory "suggests a role for media in deciding what issues the public is aware of," second level agenda-setting theory suggests "the media also frame attributes of these issues; thus, affecting how the issue is defined" (Coleman & Banning, 2006, p. 313). Agenda-setting research conducted by Coleman and Banning (2006) examined television news' framing of presidential candidates during the 2000 election. They noted that in examining second level agenda-setting most past research had not considered non-verbal, visual aspects. Coleman and Banning (2006) proposed to close the gap between analysis of verbal and nonverbal representations of candidates given the "adeptness of nonverbal attributes in communicating affective information" (p. 313). They argued what the audience sees is as influential as what it hears. "Stories are often complex combinations of visual and verbal content, and in some instances the visual information is so powerful that the verbal attributes can be overwhelmed" (Coleman & Banning, 2006, p. 314). This is important for the present study because what is said in the news coverage through reporters and anchors must be weighed against what is shown in the video to represent Middle Eastern women.

Frame-setting, or the act of assigning attributes within a story to newsmakers, has been a distinct area of study that has been progressively merged into agenda-setting studies (Aday, 2006, p. 728). This is due to the expansion of agenda-setting research to include first-level and second-level agenda-setting. News selection is at the heart of agenda-setting because the issues that fail to pass through the gatekeepers of the news also fail to give the public salient cues regarding the relative importance of the issues (Aday 2006). Audiences overlook those issues that do not make the final cut for broadcast due to lack of exposure. Attributes newsmakers assign to issues influence the perceptions the public links to the newsmakers (in this case, Middle Eastern women). Therefore, while first-level agenda-setting postulates that the media influence what we think about, the second-level of agenda-setting postulates that the media does, in fact, influence how we think (Wanta, Golan, and Lee, 2004).

Wanta, Golan and Lee (2004) found that exposure to news coverage of foreign nations can influence individuals' perceptions of those nations. The more negative news coverage a nation received the more negative the public opinion of that nation became. Further, there was no correlation between positive news coverage and positive public opinion. That is, once a negative perception was developed, positive coverage did not offset initial, negative perceptions. Negative attributes of such representations influenced the public's perception of

candidates in the 2000 presidential election. Al Gore exhibited more positive nonverbal expressions than George W. Bush in media coverage (Coleman & Banning, 2006). Consequently, Gore made viewers feel more hopeful and proud. Bush made subjects feel more angry and afraid. Bush was significantly more likely to be perceived as dishonest and out of touch with ordinary people. Not only were the differences significant, but the public opinion was significantly correlated with exposure to candidates' nonverbal expressions. However, Bush did win the presidential race, which indicates that opinions can change over time and that public opinion does not necessarily correlate with definitive action. While Gore received the largest popular vote, and the election was contested, if citizens had voted for Gore as overwhelmingly as Coleman and Banning's (2006) results of subjects' responses to his visual representation implied they would have, the vote would not have been close enough to contest.

Wanta, Golan, and Lee (2004) and Coleman and Banning (2006) coded for valence in their research. Valence is defined by Coleman and Banning (2006) as rating "on the basis of positive, negative, neutral" (p. 318). Second-level agenda-setting operates through effective framing, or assigning attributes to a newsmaker. Therefore, the attributes assigned are done so through positive, negative or neutral portrayals (p. 315). Wanta, Golan, and Lee (2004) defined valence as whether a country or nation "was covered in a predominantly positive, negative, or neutral manner" (p. 370). Both studies developed specialized methods of determining positive, negative, and neutral coverage based on the focal attributes the research intended to measure.

The unit of analysis in research by Coleman and Banning (2006) was the individual camera shot of candidates on television. Therefore, a new operationalization of coding for valence of visual attributes was required. Nonverbal behaviors were characterized using five nonverbal dimensions of activity, posture, arms, hands, and eyes. They also used verbal attributes in which binary words such as angry versus proud and hopeful versus afraid were used to measure valence. Wanta, Golan, and Lee (2004) developed a system of determining negative coverage as including reports that a foreign country was involved with activities not in alignment with the goals or interests of the United States. It is important to note that Wanta, Golan, and Lee's (2004) research transferred the object/newsmakers ideology of agenda-setting and applied it to a country or a nation's coverage in the news. Presumably, agenda-setting can be applied to a group of people (Middle Eastern women). The second-level agenda-setting theory still applied, despite the fact that the object of research was a country and not an object/individual.

### *Literature on Western Portrayals of Middle Eastern Women*

Quantitative research on portrayals of Middle Eastern women, or women in an Islamic society, has been limited in Western media. Fahmy (2004) conducted one of the few content analyses involving Middle Eastern women (Afghani women). She examined Associated Press photographs prior to and

after the fall of the Taliban regime and found that more positive imaging of Afghan women occurred after the fall of the Taliban regime. Qualitative research has been conducted discussing the power of the veil in Western perception (Macdonald, 2006), the role of Middle Eastern women in their societies, and portrayals of Middle Eastern women as gendered slaves in need of "saving" by the West (Ayotte & Husain, 2005, p. 112).

Kahf (2006) observed that the Western prototype of Muslim women is one of victimized categories. If the women manage to avoid the victim stereotype, they are most often relegated to the assignment of escapees. Victims are classified using seven aspects that may be used in Western media to portray the Middle Eastern woman (Kahf, 2006). Those aspects include references to culture, family, and behavior (see Table 1). The escapee frame uses the same representations, except the escapee also battles her birthright, owes gratitude to the West for setting her free from oppression, and removes the veil. The escapee may also begin to ascribe to mainstream sexual values in Western society, as well as develop Zionist sympathies (Kahf, 2006).

Table 1

*Victim/Escapee Aspects*

Aspects	Victim	Escapee
Mute Marionette	X	X
Meek Matrons	X	X
Forbidding Father	X	X
Cruel Country	X	X
Vile Veil	X	X
Rotten Religion	X	X
Stifled Sexuality	X	X
Rejection of Religion	---	X
Rejection of Veil	---	X
Sexually Liberated	---	X
Rescued by the West	---	X
Battler of Birthright	---	X

Jiwani (2005) found evidence that Arab/Muslim women are depicted as victims in her content analysis of Montreal's *Gazette* newspaper. However, she also discovered a contradictory frame pervading news coverage regarding women: the perspectives of victim and threat. She noted the profile builds a "confusing and sometimes contradictory conflation of woman as helpless victim and manipulative activist" (Jiwani, 2005, p. 186) and argued that Middle Eastern women's activist efforts still support an "us" versus "them" paradigm.

One of Kahf's (2006) victim cues is the vile veil. Much prior research has found that the "veil" is used as a means for

Western media to frame Middle Eastern women as oppressed and submissive. The image of the Middle Eastern woman is often as a "veiled, uneducated, oppressed, silent female" (Hamilton, 1994a, p. 175). While there are some who dispute this image, it remains a dominant perspective for many.

"There seems to be considerable agreement that the *burqa*...has become the universal symbol of women's oppression in Afghanistan" (Ayotte & Husain, 2005, p. 115). Ayotte and Husain (2005) argued as a result of the fetishization of the veil in western societies, *burqa*-clad figures have become the visual and linguistic signifier of Afghan women's oppression. Fahmy (2004) supported this argument, noting that in Western thought the *burqa* is a visual element that conveys submission.

Analysis of Muslim women within different regimes is "inhibited by a fixation on veiling" where the veil refuses "the play of difference" that exists for Muslim women across the Middle East (Macdonald, 2006, p. 7). Following September 11, 2001, a plurality of women's voices was accepted in more liberal areas of the media, but this was undermined by the continuing obsession with veiling/unveiling in Western discourse (Macdonald, 2006). Macdonald (2006), like Ayotte and Husain (2005), found that the veil has become an "all-encompassing symbol of repression, and in its dominant association with Islam reinforces the monocular representation of that religion" (p. 8). The veil also has the capacity to evoke a plethora of emotions including fear, hostility, and derision in those outside the Middle East (Macdonald, 2006).

After the fall of the Taliban government, some Western officials (including President George W. Bush) expected Afghan women to rejoice, unveil themselves, and embrace liberation from the burqa mandate. The reality was that Afghan women continued to wear the burqa, despite its no longer being required by the government (Fahmy, 2004). In her content analysis, Fahmy (2004) examined visual subordination, imaginary contact, point of view, social distance, and behavior shown in AP photographs to determine if stereotypes prevail in press photographs. Visual subordination was based on the principle that the burqa was understood by Western audiences to be an icon of oppression. Therefore, images of women in burqas were coded as visually subordinating Afghan women. Contact implies that the viewer and the Afghan woman photographed can establish contact through eye direction. If subjects in photographs are depicted as begging, pleading or asking for help, they are understood by the viewer to be submissive, passive and with little power over their lives (Fahmy, 2004, p. 94).

Fahmy (2004) used point of view and social distance to determine if Afghan women were depicted in stereotypical frames in the photographs. Point of view implies the camera angle at which the photo was taken. If the photo was taken from above, it implies symbolic empowerment and superiority of the viewer. If the image is shot at eye level, it implies equality, and shots from below imply that the viewer is inferior and/or submissive to the subject. Social distance involves where the woman is in the picture. Medium- to close-range shots signify

individuality of the subject and build a relationship between the viewer and the subject. The opposite is equally true with shots from long distances conveying the women as strangers.

Fahmy (2004) studied behavior, including physical activity and general portrayal, as an indication of whether Afghan women were pictured in subordinate traditional roles. Physical activity is further sub-divided between passive and active physical activity. Passive would include the woman's simply sitting or standing without completing a task. Active would illustrate the woman's working to accomplish something a goal. The general portrayal aspect of Fahmy's (2004) research distinguished between women depicted in traditional roles and nontraditional roles in society. Traditional roles included images of women working inside the house, acting in a motherly role, carrying a child or food, etc. Non-traditional roles would include working outside the home, interacting with friends, or shopping as modes of behavior outside the traditional roles (Fahmy, 2004, p. 98).

Fahmy (2004) also examined the mute marionette attribute in her study. The mute marionette aspect was defined by Kahf (2006) as a Muslim woman portrayed as "powerless to speak" (p. 79). The West must give her a voice. The assignment of Muslim women as oppressed fixes their status as objects by denying them "the power to speak of differences" (Ayotte & Husain, 2005, p. 118). Further, Western media have problematically ventriloquized Muslim women by entering into "discourses speaking for (both on behalf of and in place of) them [Afghan women]" (Ayotte & Husain, 2005, p. 116). Saliba (1994) reported that not only are women silenced in media portrayals, but they are often rendered absent. The absent Arab woman occurs in two forms. "The first is a literal absence, when the Arab woman is not present or is entirely missing from the scene; the second, a symbolic absence, when she is present but only for the purpose of representing her invisibility or silence" (Saliba, 1994, p. 126).

## Research Questions

Based on past research, there is a need to examine broadcast television news coverage of Middle Eastern women in order to add to the body of research and literature currently available. Hamilton (1994a), Wilkins (1995), and Fahmy (2004) have investigated the prevalence of negative portrayals of Middle Eastern women in the West in print and have found that the largely negative portrayals of Middle Eastern women in a Western context results in a negative stereotype applied to Middle Eastern women. Therefore, this research seeks to answer three research questions:

**RQ1:** How are portrayals of Middle Eastern and Islamic women (e.g., portrayals of the veil, contact, behavior, point of view, social distance, and victimization) distributed in major network (ABC, CBS, NBC) and cable (CNN and Fox News) television news coverage?

**RQ2:** Does American television news coverage portray Middle Eastern women positively, negatively, or neutrally?

**RQ3:** Do certain aspects of portrayals in television news coverage (e.g., portrayals of the veil, contact, behavior, point of view, social distance, and victimization) correlate more frequently with negative portrayals of Middle Eastern women?

## Methods

Quantitative and qualitative research techniques were employed to examine the research questions, including a content analysis of news coverage on American network and cable television news.

### Quantitative Approach

The content analysis consisted of coding for factors to indicate valence in coverage of Middle Eastern women in television news. News packages, defined by Shook (2005) as "an edited, self-contained report of a news event with pictures, sound bites, voice-over narration and natural sounds, typically lasting from 1:10 minutes to 3:30 minutes for features" (p. 187) were the units of analysis. These news packages were selected through convenience sampling from the Vanderbilt University Television News Archives. Riffe, Lacy and Fico (1998) reported the archive is often used in television news research because it aids in "locating stories about given topics" (p. 204). The specific nature of this research, analyzing only Middle Eastern women's portrayals, lends itself to this archive selection method.

Sixty-one video packages meeting the date and time limit parameters of this study were selected from the database using a Boolean content search. Stories airing after September 11, 2001 and prior to December 31, 2008, were analyzed because prior research (Fahmy, 2004; Jiواني, 2005) claimed portrayals of Middle Eastern women became more negative after September 11, 2001.

ABC, CBS, and NBC are the three largest networks. In 2007, ABC's World News Tonight reported an average audience of 8.38 million. NBC averaged 8.29 million viewers, while CBS ranked third, averaging 6.43 million (Project for Excellence in Journalism, 2008). Fox News and CNN are the primary cable news providers in the United States. Fox News had a median primetime audience of 1.41 million; CNN reported a median prime-time audience of 736,000 in 2007 (Project for Excellence in Journalism, 2008).

### Qualitative Approach

Within the video, both the news package and the anchor lead-in were analyzed. The anchor lead-ins were analyzed based on research by Reese and Buckalew (1995). Following their example, anchor lead-ins were discussed on a qualitative basis, because they employ adjectives and sensationalized wording, which can reveal frames more directly (Reese & Buckalew, 1995).

### Variables

The Middle East is not well defined as a geographic location or ethnic group. Afghanistan is particularly difficult to place in scholarship. Some consider it beyond the "boundar-

ies” of the Middle East, placing it in South Asia (The World Bank, 2009). However, media outlets, such as The Washington Post, often lump Afghanistan into the Middle East. Therefore, Afghanistan is incorporated because of its inclusion in some Western mass media based on its Islamic society and its relationship with the United States as an area of conflict. News stories analyzed in this research included women from Afghanistan, Iraq, Palestine, and Saudi Arabia.

Portrayals of Middle Eastern women in television news were defined through the use of the veil, contact, portrayal, point of view, social distance, presence, victimization, and behavior. Valence (positive, negative, or neutral) of portrayals was determined by examining these factors. News packages were ranked through ordinal codification by overall depiction as 1 = positive, 2 = neutral, and 3 = negative in accordance with content analysis procedure. Valence was determined using coding factors. If positive aspects outnumbered negative aspects, the story was defined as Positive. If negative aspects outnumbered positive aspects, the story was coded as Negative. In the instance that negative and positive aspects were equal, the story was coded as Neutral.

For Middle Eastern women’s portrayals, Middle Eastern dress was coded per instance using a nominal coding system. For the purpose of this study only *burqas*, *hijabs*, and *niqabs* were coded for as the most common types of coverings for the countries in the analyzed packages. Watson and LeJeune (2004) define the *burqa* as “a long, loose garment covering the entire face and body with a mesh material, allowing for some visibility” (p. 8). The *hijab* “can mean anything from modest Islamic dress to a full veil. It is often a headdress that covers a woman’s hair in combination with long, loose clothing” (Watson & LeJeune, 2004, p.7). The *niqab* is “a veil worn by women that completely covers the face” (Watson & LeJeune, 2004, p. 8). The veil was coded as 0 = absent, 1 = present/positive, 2 = present/neutral, and 3 = present/negative. Determinations of valence as positive, negative, and neutral were determined considering the verbal, contextual, and visual usage of the veil in the portrayal. For instance, if the script noted the important function of the *hijab* in signaling women’s Muslim faith, and the image accompanying it was one of a *hijab*-clad smiling as she praised the veil in an interview, this was coded as present/positive. This assured stories containing veil cues were not assumed to be negative simply due to the presence of these articles of clothing and provided a method of recognizing patterns between dress and valence.

Visibility was based on Saliba’s (1997) research in which Middle Eastern women seemed to be absented. If the story referred to Middle Eastern women, but none were shown, it was coded as absent=0. The contact factor was also coded using this binary system, based on whether the women shown in the videos of news packages made eye contact with the viewer. Based on Fahmy’s (2004) research, women shown wearing face veils or *burqas* in which the eyes cannot be seen were coded as no contact.

Victimization was determined based on Kahf’s (2006) victim/escapee aspects. Each aspect was coded using the binary system of presence. This method is useful in examining how certain aspects are used in conjunction with one another and the frequency of certain aspects. It also allows for data manipulation at the interval level, providing more information in data analysis. Victimization includes the factors that create the Escapee category as noted by Kahf (2006). Therefore, coding victimization was divided between victim and escapee aspects, and stories were coded as either Victim or Escapee.

Behavior was divided between physical activity and general portrayal (Fahmy 2004) and was coded through binary coding. Physical activity was passive or active, and general portrayal was either traditional or non-traditional. Point of view was coded on the three-step scale of valence, in which shooting from above indicates submission (Fahmy 2004) and was coded as 3 = negative. Those images of women shot at eye line were coded as 2 = neutral, and those from below were 1 = positive. Social distance, or placement in the photograph, was coded similarly. Therefore, shots with Middle Eastern women in the foreground were positive, while those with the woman in the background were negative. Women depicted in the middle of the frame were coded as neutral.

An independent coder was employed to enhance objectivity. Twenty percent of stories were coded (Riffe, et. al, 1998). Stories to be coded were selected through simple random sampling. Inter-coder reliability was measured for each variable using the Scott’s Pi reliability formula. All variables were above the acceptable .70 standard of reliability (see Table 2).

## Results

### Research Question 1

The first research question asked, “How are portrayals of

Table 2

Intercoder Reliability

Variable	Intercoder Reliability
Arab Female Presence	1.00
Contact	1.00
Behavior	.75
Portrayal	1.00
Point of View	.80
Social Distance	.75
Burqa	.84
Hijab	1.00
Niqab	1.00
Mute Marionette	.80
Meek Matrons	.82
Misogynist Males	.80
Radical Religion	.75
Cruel Country	.80
Vile Veil	.80
Stifled Sexuality	.75
Rejection of Religion	1.00
Battler of Birthright	.80
Rescued by the West	1.00
Rejection of the Veil	1.00
Sexually Liberated	1.00

Middle Eastern women distributed in major network and cable television news coverage?" Data analysis showed that women were present in 98% of stories. However, contact was only depicted in 20% of the stories; women were active in 83%. Further, portrayals of women as non-traditional were more than three times as frequent (78%) as traditional portrayals (22%). Both point of view and social distance were largely neutral at 76%. However, negative portrayals (22%) outweighed positive portrayals (2%) in point of view and social distance (15% negative; 9% positive).

The burqa was absent in 57% of the stories, present-neutral in 3% and present-negative in 40% of the stories. The hijab was absent in 15% of stories, present-positive in 3%, present-neutral in 60%, and present-negative in 22% of the stories. The niqab veil was absent in 78% of stories. When it was depicted, there were no instances of it being present-positive, and representations of present-neutral consisted of 10% of stories while present-negative constituted 12%. The burqa was never depicted as present-positive and was present-negative in all of the stories (23) depicting women in Afghanistan. Kahf's (2006) victimization aspects were analyzed, and all but one of 61 stories (98%) used at least one victim/escapee aspect. Eighty-four percent of stories were characterized as victim, and 14% were escapees (see Table 3).

#### Research Question 2

Research question two asked, "Does American television news coverage portray Middle Eastern women positively, negatively, or neutrally?" More stories were negative (53%) than positive (34%). Neutral coverage constituted 13% of the

Table 3

Victim/Escapee General (N = 61 stories)

Victim/Escapee Aspects	Featured (in stories)	
	Nos.	%
Mute Marionette	21	34%
Meek Matrons	30	49%
Misogynist Males	46	75%
Radical Religion	49	80%
Cruel Country	57	93%
Vile Veil	27	44%
Stifled Sexuality	19	19%
Rejection of Religion	1	2%
Battler of Birthright	6	10%
Rescued by the West	4	7%
Rejects the Veil	1	2%
Sexually Liberated	0	0

stories analyzed. However, anchor lead-ins must be considered in determining valence. As the anchor introduction to the story sets the tone for what is to follow, it is interesting that the majority of anchor lead-ins used one of two frames. The first frame is a "before the U.S. interceded and after" frame. The second is a "terrorist/religious fanatic" frame.

For stories on countries where the U.S. was actively engaged, women were most frequently depicted in the anchor lead-ins as victims of their culture in need of rescue or beneficiaries of American intervention. For example, Tom Brokaw introduced an in-depth NBC report on Afghan women in October 2001.

**Brokaw:** *NBC News in-depth tonight, the other victims of terror. Women in Afghanistan and girls held hostage, in effect by the Taliban regime. Tonight's question was 'What is the life expectancy of an Afghan woman?' The answer: it's just 45 years. Not surprising when you know about the way they are forced to live. NBC's Ron Allen has our in-depth report. This is a rare look behind the veil.*

Introductions tended to invoke the idea that women were victims of their society, unable to change their status without Western intervention. Even once the Taliban was removed, the idea that Afghanistan might continue to persecute women prevailed. Stories were analyzed across the time frame to determine that this was a portrayal that prevailed from 2001 to 2008.

Palestine is somewhat unique in that the U.S. is not militarily engaged in the Palestinian-Israeli conflict. However, it is one of the few nations (although not a nation-state) other than Iraq and Afghanistan featured in coverage of Middle Eastern women in this sample. News stories about Palestine portrayed women most frequently as terrorists and as victims. In February 2002, Aaron Brown introduced a CNN story regarding suicide bombers in Palestine:

**Brown:** *Twice now suicide bombers, terrorists entering Israel, have been women. That is unheard of. We can all speculate on what it means, but we can't ignore it as a fact. And the fact is that these two women are being hailed as something akin, at least, to feminist heroes in the Arab world.*

Brown states that Palestinian women acting as militants or suicide bombers are "unheard of" (CNN, February 28, 2002). However, Najjar (1992) noted that Palestinian women "actively joined in the 1976 and 1981 uprisings, but they joined in even larger numbers in the 1987 Intifada." (p. 85). Therefore, Palestinian females have a well-documented history of militancy.

#### Research Question 3

The third research question asked if certain aspects of portrayals correlated more frequently with negative portrayals of Middle Eastern women. Comparisons of negative news coverage to portrayal factors implied that negative depictions did frequently occur alongside specific depictions of the burqa,

victim aspects, and other portrayal factors.

When the burqa was present-negative, the meek matron victim aspect was employed more frequently than expected (see Table 4). A chi-square test revealed that when burqas were present-negative women were significantly more likely to be depicted as meek matrons,  $X^2(1, N=58) = .018, p < .05$ . When the burqa was absent, the meek matrons aspect was more likely to be absent as well. Stifled sexuality aspects also seemed to occur more frequently than expected when the burqa was present-negative. When the burqa was absent, stifled sexuality was recorded in fewer instances than expected (see Table 5). A chi-square revealed that women were significantly more likely to be depicted as victims of stifled sexuality when the burqa was depicted as negative and less likely when the burqa was absent,  $X^2(1, N=58) = .004, p < .05$ .

Other factors of portrayal could not be subjected to chi-square tests, but the frequencies were compared to examine

Table 4

*Burqa Absent-Negative and Meek Matrons Chi-Square Test (N=58) with 0 = Absent and 1 = Present*

			Burqa - Absent or Negative		Total
			Absent	Present / Negative	Absent
Meek Matrons	0	Count	22	8	30
		Expected Count	17.6	12.4	30.0
	1	%	73.3%	26.7%	100.0%
		Count	12	16	28
Total		Expected Count	16.4	11.6	28.0
		%	42.9%	57.1%	100.0%
		% of Total	20.7%	27.6%	48.3%
		Count	34	24	58
		Expected Count	34.0	24.0	58.0
		%	58.6%	41.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.545(b)	1	.019		
Continuity Correction(a)	4.360	1	.037		
Likelihood Ratio	5.635	1	.018		
Fisher's Exact Test				.032	.018
Linear-by-Linear Association	5.450	1	.020		
N of Valid Cases	58				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.59.

relationships that could be analyzed in future research, including levels of victimization comparisons with activity, point of view, and behavior and frequency of portrayal factors/aspects most frequently used in negative depictions. The victim/escapee index was divided into three subgroups: below mean (less victimization), mean (average victimization) and above mean (most victimization). Data showed women were more frequently depicted as active, even as they were depicted as victims. With respect to portrayals (either traditional or non-traditional), women were depicted as non-traditional more frequently when they were depicted as victims (see Table 6). Point of view was largely neutral; in all three categories neutral point of view occurred more frequently than expected. Negative point of view depictions occurred less frequently than expected in all three categories. The victim/escapee index comparisons with social

Table 5

*Burqa Absent-Negative and Stifled Sexuality Chi-Square Test (N=58) with 0 = Absent and 1 = Present*

			Burqa - Absent or Negative		Total
			Absent	Present / Negative	Absent
Stifled Sexuality	0	Count	28	11	39
		Expected Count	22.9	16.1	39.0
	1	%	71.8%	28.2%	100.0%
		Count	6	13	19
		Expected Count	11.1	7.9	19.0
		%	31.6%	68.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.518(b)	1	.004		
Continuity Correction(a)	6.941	1	.008		
Likelihood Ratio	8.573	1	.003		
Fisher's Exact Test				.005	.004
Linear-by-Linear Association	8.371	1	.004		
N of Valid Cases	58				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.86.

distance portrayals were found to be largely neutral as well. However, negative portrayals in the above mean category were higher than expected, implying that when women are depicted most negatively, they tend to also be distanced from the viewer (see Table 6).

Table 6

*Distribution of Victim/Escapee Index in 3 Categories and Portrayal Factors (N=60)*

	Below Mean				Mean				Above Mean			
	Expected No.	%	Observed No.	%	Expected No.	%	Observed No.	%	Expected No.	%	Observed No.	%
<b>Contact</b>												
No Contact	15.2	25%	15	25%	12.8	21%	14	23%	20	33%	19	32%
Contact	3.8	6%	4	7%	3.2	5%	2	3%	5	8%	6	10%
<b>Portrayal</b>												
Traditional	4.1	7%	3	5%	3.5	6%	3	5%	5.4	8%	7	28%
Non-Traditional	14.9	25%	16	27%	12.5	21%	13	22%	19.6	33%	18	30%
<b>Behavior</b>												
Passive	3.2	5%	3	5%	2.7	5%	1	2%	4.2	7%	6	10%
Active	15.8	26%	16	27%	13.3	22%	15	25%	20.8	35%	19	32%
<b>Point of View</b>												
Positive	0.3	0.1%	0	0%	0.3	0.1%	0	0%	0.4	0.1%	1	2%
Neutral	14.6	24%	18	30%	12.3	21%	13	22%	19.2	32%	15	25%
Negative	4.1	7%	1	2%	3.5	6%	3	5%	5.4	9%	9	15%
<b>Social Distance</b>												
Positive	1.6	3%	2	3%	1.3	2%	1	2%	2.1	3%	2	3%
Neutral	14.6	24%	14	23%	12.3	21%	14	23%	19.2	32%	18	30%
Negative	2.9	5%	3	5%	2.4	4%	1	2%	3.8	6%	5	8%

The average number of negative aspects used when the overall story was negative was four. A comparison of Less Negative (below the mean), Moderately Negative (at the mean), and More Negative (above the mean) revealed that the largest portion of stories (44%) were Less Negative and used 1-3 aspects (see Table 7). That is, while they were more negative than positive, they used less than the average number of negative aspects in their portrayals. The More Negative stories were slightly more common (30%) than the Moderately Negative (26%).

The comparison of stories coded as Negative with those stories coded as Victim seemed to imply that when the story was More Negative the meek matrons, radical religion, vile veil, and stifled sexuality aspects occurred more frequently than expected (see Table 8). However, the misogynist males aspect and the cruel country aspect were most frequent in those



stories that were categorized as Less Negative. Therefore, these two aspects were featured most frequently when women were depicted as less victimized, but they were key aspects to building this victimized frame.

## Discussion of Results

In past research it has been alleged that American media portray Middle Eastern women and their society negatively. Overall, data analysis seemed to imply that news stories were more negative than positive in their depictions of Middle Eastern women. The results of this research support past research of Middle Eastern women's depictions in print news.

Table 7

*Distribution of Negative Aspects (N=61)*

		Frequency	Percent
Valid	Below Mean	27	44.3
	Mean	16	26.2
	Above Mean	18	29.5
	Total	61	100.0

These results seem to imply that, as Fahmy (2004) argued, American news media prevent contact between the Middle Eastern women being depicted and viewers. The data support Fahmy's (2004) argument that the burqa is a negative symbol to signify women's oppression in Afghanistan. The burqa was never depicted as positive in any story, and in all stories about Afghan women it was depicted as negative. It appears the burqa serves as a concrete symbol of women's oppression in Afghanistan in American television news coverage, although this has been interpreted in various ways in academic literature. This requires consideration regarding stories when it was depicted present-neutral. If the burqa is repeatedly signified

Table 8

*Victim/Escapee Aspects by Negativity (N=61)*

	Less Negative				Moderately Negative				More Negative			
	Expected No.	%	Observed No.	%	Expected No.	%	Observed No.	%	Expected No.	%	Observed No.	%
<b>Weak matrons</b>												
Absent	13.7	23%	18	30%	8.1	13%	6	10%	9.1	15%	7	12%
Present	13.3	22%	9	15%	7.9	13%	10	17%	8.9	15%	11	18%
<b>Radical religion</b>												
Absent	5.3	9%	9	15%	3.1	5%	1	2%	3.5	6%	2	3%
Present	21.7	36%	18	30%	12.9	21%	15	25%	14.5	24%	16	26%
<b>Vile veil</b>												
Absent	15	25%	29	48%	8.9	15%	10	17%	10	17%	4	7%
Present	12	20%	7	12%	7	12%	6	10%	8	13%	14	23%
<b>Stifled Sexuality</b>												
Absent	19	31%	22	36%	11	18%	11	18%	12	20%	9	15%
Present	8	13%	5	8%	5	8%	5	8%	6	10%	9	15%
<b>Misogynist Males</b>												
Absent	7	12%	9	15%	4	7%	2	3%	4	7%	4	7%
Present	20	33%	18	30%	12	20%	14	23%	14	23%	14	23%
<b>Cruel Country</b>												
Absent	2	3%	2	3%	1	2%	1	2%	1	2%	1	2%
Present	25	41%	25	41%	15	25%	15	25%	17	28%	17	28%

as a symbol of oppression, regardless of what textual or oral representations are presented, can the burqa ever truly be understood as a neutral symbol?

The hijab, on the other hand, was depicted in 63% of the stories as either positive or neutral. This seems to imply that news agenda-setters view the hijab as a more acceptable veiling practice. In 2002, the Brookings Institute reported that there were 3 million Arab Americans living in the U.S. and 6 million Muslim Americans who could be watching these news programs (Telhami, 2002). As such, the need to "other" women who wear the hijab may be less, because the hijab has ties to certain American populations.

Unlike the findings of Fahmy (2004), point of view and social distance were most frequently depicted neutrally. However, the data seem to imply a depiction is more likely to be negative if it is not neutral. One must consider the nature of television news in analyzing these two variables. Fahmy's (2004) analysis was of photographs, or single moments captured in time. In this study, television stories were analyzed that ran from 1:30 to 3:30 minutes. The typical television journalist has a larger "blank canvas" to fill with content, allowing for a larger variety of shots within a story. Therefore, the point of view and social distance variables may not reflect agenda-setting or frame-setting in television as much as they reflect the nature of the television news product. The need for diverse shots and a variety of distances/angles in television coverage may make these two variables inappropriate for analyzing agenda-setting in television news.

Kahf (2006) postulated that Middle Eastern women are most frequently depicted within the victim/escapee paradigm. The results of this content analysis did seem to support her theory. None of the stories, as Kahf (2006) predicted, mentioned any grassroots efforts within the region against human rights violations or for feminist equality in the region prior to Western intervention.

A qualitative analysis of the stories suggests that Jiwani's (2005) postulation that Middle Eastern women are depicted simultaneously as victims and threats could be supported, under certain circumstances. Stories about Palestinian women all featured them as threats: either as nationalist activists for Palestine against Israel and the United States' foreign policy or as fanatical religious martyrs. However, these women were also depicted as victims and other Middle Eastern females were also depicted as threats. For example, in stories concerning Iraq, women's suicide bombings are explained as terrorist tactics luring women in, against their will or by exploiting their ignorance. Anchor lead-ins tended to use tones of bafflement and shock in relation to Iraqi female bombers, a different use than anchor lead-ins regarding Palestinian female bombers.

## Recommendations for Further Research

The importance of news coverage is that it conveys messages to the viewing/reading public. Thus, one must wonder if news coverage influences public perception of the issue being covered. The present study could not investigate this particular

question. However, it can serve as a starting point to suggest further research regarding portrayals of Middle Eastern women in television news. Future research should inquire about the effects of those portrayals on the public perceptions of Middle Eastern women as portrayed in American television news. Future focus group research could examine stories, after being determined to be positive, negative or neutral, that are shown to individuals with a variety of preconceptions about the state or "plight" of Middle Eastern women in their societies. Using a larger sample could improve the knowledge of television news portrayals of Middle Eastern women, including biases (if any) across networks and cable channels. For this research, date/time parameters, story availability in the Vanderbilt News Archives, and limited research funds necessitated a smaller sample for this initial content analysis. However, a larger sample might reveal more prominent patterns of cues and frame-setting, allowing for more sophisticated statistical techniques in analyzing the data.

### Conclusion

This is the first research of its kind, examining the portrayals of Middle Eastern women in American television news coverage. These results serve to build a foundation on which future research can be based. The data imply that American television news does portray Middle Eastern women more negatively than positively. Women are frequently depicted as victims, as Kahf (2006) argued. Contact between the Middle Eastern women depicted is often not observed, as Fahmy (2004) found. However, unlike Saliba's (1997) argument, it cannot be concluded that American television news "absents" Middle Eastern women; women were present in 98 percent of stories. Unlike the findings of Fahmy (2004), point of view and social distance were both largely neutral. However, this may have more to do with the conventions of television news gathering rather than agenda-setting.

The data do seem to support Shirazi's (2001) argument regarding the burqa. The burqa was never depicted as positive, and when it was depicted, it was typically depicted as negative rather than neutral. Therefore, the burqa does seem to serve as a symbol of oppression for American audiences in describing Afghanistan's Islamic society. Behavior was generally non-traditional. Women were more often depicted as active, though they were simultaneously cast as victims or escapees.

Overall, the results suggest that American television news and cable network channels do employ a form of agenda- and frame-setting, depicting Middle Eastern women as victims of their cultures. Differences in experience are often ignored, replaced with a generalized depiction of an existence of oppression. The burqa remains a pervasive symbol of the misogynistic, patriarchal society in Afghanistan. On the other hand, the stories depict Afghan and Iraqi women as having more rights and freedom, thanks to American intervention in overthrowing the regimes that relegated women to second-class citizens. However, both Iraqi and Palestinian women were depicted as new terrorist threats and were often victimized as sacrificial

This research was intended to determine the portrayals of Middle Eastern/Muslim women that prevailed following September 11, 2001, in American television news coverage. While initial impressions have been gathered and certain patterns seemed to occur, further research can only help our knowledge in understanding the mediated images of Middle Eastern women in television news coverage. Questions regarding bias of individual networks, correlations between coding factors, and their impact on overall portrayals may be discovered in further research with larger sample sizes than this research could provide. However, this research does provide a foundation on which further research may build upon to answer these remaining questions

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#### Mentor Comments:

Professor Hoyt Purvis draws attention to the unique contribution of Marci Manley's research on television coverage of Middle Eastern women, noting the way in which she drew from multiple disciplines in order to answer the challenging questions she posed.

Marci Manley is an exceptional student and an exceptional young woman who has been undaunted by the challenges of pursuing a demanding, innovative, and ground-breaking research project. Her research project effectively combines her interests in journalism, Middle East studies, and international relations. This was a project which she conceived and which she pursued independently. While Marci stayed in regular contact with me and also sought advice from other faculty members, this was her project from start to finish.

All the work that she did demonstrated a seriousness of purpose and commitment that was highly impressive. Marci had developed a special interest in the way that news is reported from the Middle East and Islamic societies, especially as it relates to women and how they are portrayed and perceived in the American media. In conceiving and carrying out this research, she was also able to draw upon valuable experiences as a study abroad student in Morocco and an intern in Washington, D. C., with Al-Arabiya, an Arab-language television network.

She thoroughly familiarized herself with the relevant, if rather limited research done on this topic as well as the pertinent concepts for both quantitative and qualitative research on media content and portrayals. She developed a good understanding of the agenda-setting and framing roles of the media and how they influence public perceptions and integrated that into her research and analysis.

What is especially distinctive and significant about Marci Manley's research is that she focused on television coverage, whereas virtually all research on coverage of Middle Eastern women had dealt with print media coverage. For obvious reasons, it is easier to study and analyze print coverage. However, Marci, who has been actively involved in broadcast journalism herself, understands the importance of television as a source of information and influence and in shaping perceptions. Therefore, she was determined to concentrate her study on televised portrayals.

It is one thing to recognize the need for and potential value of such an analysis; it is quite another to be able to pull it off. In this case, it meant painstaking collection from the Vanderbilt Television Archives of relevant video segments. Simply identifying, selecting, and assembling the video clips and transcripts is a major undertaking. However, that was only a part, if an integral part, of Marci's project.

Her study focused on whether the coverage presented a more positive or negative image and the extent to which it reinforced stereotypes of Middle Eastern women. Using recognized content analysis techniques, tailored to the specific needs of this project, she developed a basis for systematic analysis of the coverage, including the lead-ins by the television news anchors. Her research, as she analyzes and explains, indicates that American television news does portray Middle Eastern women more negatively than positively.

Her work represents the first serious examination of the television portrayals of women from the Middle East and Islamic societies and in that sense is definitely ground-breaking and unique. This project is original, the analysis is sophisticated, and the overall product represents a significant contribution to research on inter-cultural portrayals and stereotypes in media coverage.

## Carbon Nanotube Cluster Based Micro-Fluidic System for Bacteria Capture, Concentration, and Separation

By Chris Nelson

Department of Biological and Agricultural Engineering

Faculty Mentor: Dr. Jin-Woo Kim

Department of Biological and Agricultural Engineering

### Abstract

*Disease-causing pathogens continue presenting enormous global health problems, especially due to their easy transmittance to people via water supply systems. The detection, filtration, and purification of bacteria-contaminated water samples are complex activities, ones subject to considerable error. Here we present a new and highly effective micro-fluidic system with carbon nanotube (CNT) clusters for effective and efficient detection, filtration, and purification of bacteria-contaminated medium. The developed system is based upon two unique properties of CNT clusters: high bacterial affinity and magnetic susceptibility. The CNTs' high affinity to bacteria cells makes them a key candidate for the bacteria adsorption. The magnetic susceptibility of their clusters allows an effective way of separating as well as containing them in the system. In this study, we designed and tested a prototype CNT-cluster based micro-fluidic system by uniquely combining the two excellent properties of CNTs. The CNT-based micro-system consisted of a micro-channel, which positions CNT clusters evenly on the bottom surface using a strong Neodymium block (1" X 1" X 1") rare-earth magnet (surface magnetic field strength = 0.684 Tesla). When bacteria suspensions were introduced, the CNT clusters in the micro-fluidic system were shown to effectively serve as bacterial adsorbing centers, which led to spontaneous adsorption and concentration of bacteria to the clusters. This was shown to happen for both types of microorganisms, i.e., Gram-positive and Gram-negative bacteria. The results demonstrate the excellent potential of the CNT based micro-fluidic system for bacteria capture, concentration, and separation.*

### 1. Background

The presence of bacteria in water systems is a danger to any operation; hence, the detection of bacterial contaminations has become a big issue. Current methods for determining the presence of bacteria include polymerase chain reaction (PCR), colony counting methods, and immunology-based methods, which have long waiting periods and are complicated processes [1]. Additionally, the problem still remains that bacteria in a dilute solution cannot be detected. In the following research, the beginnings of a method are suggested for determining the presence of particular pathogens and reducing the concentration requirements 107 – 104 colony forming units (CFU)/ml to close to 81 CFU/mL [1].

The advent of nanotechnology has opened many doors for sensing in the nano-scale. Nanotechnology has the ability

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to revolutionize molecular electronics, medical chemistry, and biomedical engineering [2]. Research has explored applications from electronics, composites, fuel cells, sensors, optics, and biomedicine [3]. Carbon nanotubes (CNTs) were first discovered in 1991 by Iijima and are cylindrical carbon rods with a diameter of 4 to 30 nm [4] and lengths up to millimeters in length [5]. The aspect ratio for such a material is enormously high at 1:28,000,000, making the CNT a very unique material with peculiar and novel properties. Multi-walled CNTs (MWNTs) are similar in structure with additional walls located around the central wall.

Recently, high affinity binding interactions between CNTs and bacteria have been demonstrated [6,7,8] and exploited for use as bacterial filters [7] and photothermal and photoacoustic antimicrobial contrast agents [8,9]. These studies demonstrate excellent potentials of CNTs as bacterial capturing agents. In addition, research by Brady-Estevéz, et al. [3] explored the use of single-walled CNTs (SWNTs) as a size exclusion filter with *Escherichia coli* K12 (a model bacterium for *E. coli* 0157:H7). They also demonstrated a method by which the SWNT system can be reused. Further, CNTs have been shown to exhibit para-magnetic and di-magnetic properties under the presence of a strong magnetic field [10]. This previous research opens the door for continued exploration of the frontier of nanotechnology by testing specifically the feasibility of a MWNT system for filtering and concentrating bacteria in water solutions by adsorption and magnetic retainment.

Parallel to research being conducted on the applications of CNTs is the work that highlights the possible toxicity they may possess. This potential drawback needs to be addressed. Warheit, et al. [11] reported that CNTs could be harmful to the pulmonary system of rats and, by extrapolation, humans. If kept in solution, the CNTs will not cause pulmonary problems. In addition, Blaise et al. [12] reported that certain nano-materials are toxic to aquatic organisms. Their research emphasized that CNTs should be retained by any system that uses them. That is where the unique magnetic properties of CNTs can be used to increase the retention of CNTs inside a system. Systems using CNTs should incorporate this concept.

Such systems would have two very distinct potential applications. The first is bacteria "filtration," functioning by size exclusion or adsorption of the bacteria. This system would need to be reusable in order to make it cost effective. The second is bacteria "concentration". The concept is that the microbes in a dilute solution become concentrated inside

a micro channel, which would provide a mechanism for more efficient bacteria detection, in particular pathogen detection. This application likely will have the greatest impact on rapid water source analysis.

The goal of this study was to build and evaluate a MWNT based system for bacteria concentration and filtration. The specific objectives included designing and fabricating an apparatus to conduct tests, determining optimal operating parameters including flow rate and MWNT concentration, determining bacterial adsorption capacity of MWNT system, and finally imaging the bacteria and MWNTs to draw conclusions.

## 2. Materials and Methods

**2.1 Bacteria Evaluation.** *E. coli* were prepared from a frozen sample (-80°C) by creating a streak plate (Luria Bertani (LB) agar) and incubating for 24 hours at 37°C. A single colony was isolated and inoculated in LB medium and then incubated in a shaker (24 hours, 37°C, 150 rpm). It was considered important during this study to know the concentration of *E. coli* cells in a suspension before experiments begin. A spectrophotometer was used to evaluate dilutions of *E. coli* suspensions in parallel to spread plating (LB agar), which generated the relation of the optical density (OD) of an *E. coli* suspension with the concentration of the suspension. The OD of the *E. coli* samples was routinely measured and the cell concentration was estimated on the basis of the empirically determined relation.

**2.2 Device Design.** Figure 1 is a graphical representation of the designed device. The device consisted of a polydimethylsiloxane (PDMS) gasket (2.5 cm × 7.5 cm × 0.2 cm) with a channel cut into the center (1.0 cm × 5.5 cm). The channel was further encapsulated by two microscope slides. The top slide had 1-mm holes drilled into the surface at opposite ends with micro-fluidic fittings attached with epoxy glue. The fittings were connected to influent and effluent tubes, which transported the sample into and out of the system. The glass slides and PDMS were further encapsulated inside an ultra-high molecular weight polyethylene (UHMWPE) clamp, which was held together with nylon bolts and nuts. This provided even pressure maintaining the seal provided by the PDMS gasket. Finally, a strong Neodymium block (1" X 1" X 1") rare-earth magnet (surface magnetic field strength = 0.684

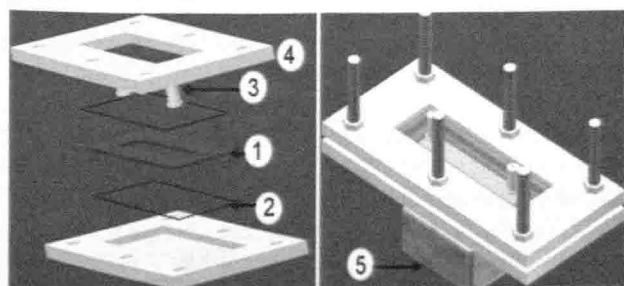


Figure 1: MWNT micro-fluidic device for experimentation 1-PDMS, 2-Glass Slides, 3-Micro-fluidic fittings, 4-UHMWPE clamp, 5-Rare-earth magnet

Tesla) was placed below the system to take advantage of the MWNT clusters' magnetic susceptibility.

### 2.3 Channel MWNT Concentration Determination.

MWNTs were injected into the system under presence of the rare-earth magnet, which caused the MWNTs to concentrate into the bottom of the channel. MWNTs were injected until the system was saturated. This was determined visually when, in the presence of the magnet, the MWNTs occupied the entire volume of the channel. The resulting MWNT concentration was selected as the optimal MWNT concentration for experimentation.

**2.4 Maximal Flow Rate Determination.** The MWNT channel retained the MWNT clusters on the basis of their magnetic susceptibility. This magnet susceptibility was of limited strength. A high flow rate could dislodge clusters from the channel. The maximal flow rate was determined on the basis of visible loss of MWNT clusters at varying flow rates.

**2.5 Adsorption Capacity of MWNTs.** To measure the capacity, a known concentration of *E. coli* was injected into the system. The effluent was collected and the concentration was measured to find the percent removal:

$$\% \text{Removal} = 1 - \frac{[E. coli]_{out}}{[E. coli]_{in}}$$

The test was conducted first by preparing a suspension of *E. coli* in LB medium. The optical density was measured to estimate the influent concentration and serial dilutions were made to the concentration that is to be tested. The system was injected with a pre-determined concentration of MWNT suspension (see section 2.3) and allowed to rest under the presence of the rare-earth magnet for 5-15 minutes. The system was then rinsed with 30 mL of water and the last few drops were collected as a negative control. Diluted cell suspensions were then injected into the system and rinsed into the collection tube. The collected samples were spread plated and incubated for 24 hours at 37°C. After incubation, the CFUs were counted and the effluent cell concentration was calculated.

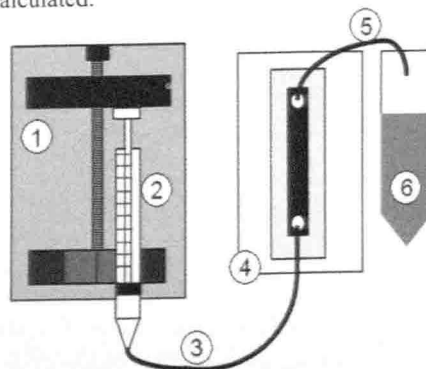


Figure 2: MWNT micro-fluidic system for estimating adsorption capacity 1- Syringe Pump, 2-10-mL Syringe, 3-Influent tube, 4-MWNT channel, 5- Effluent tube, 6-Collection for testing



**2.6 Imaging.** Epi-fluorescence microscopy was used by staining the E.coli with LIVE/DEAD BacLight™ Bacterial Viability Kit (Invitrogen, Carlsbad, CA). The kit contained two nucleic acid stains: green-fluorescent SYTO® 9 stain and a red-fluorescent propidium iodide stain. SYTO 9 (green) labeled both live and dead bacteria when used alone. Propidium iodide (red) penetrated bacteria with damaged membranes (dead bacteria). The images were made with a Light Microscope (Axioskope 2 Plus, Carl Zeiss, Inc., Germany) equipped with 12-bit Color MicroImager II cooled digital camera (QImaging, Burnaby Canada). The light microscopy system was additionally equipped with filter sets consisting of band-pass filters covering 450 to 490 nm (fluorescein isothiocyanate (FITC)) and 512 to 546 nm (propidium iodide (PI)) for excitors and absorbance filters covering wavelengths beyond 515 nm (FITC) and 590 nm (PI) to acquire epi-fluorescence images for the stained cells. Imaging the MWNT system with infused E. coli showed the location relative to the MWNT clusters as well as their health.

### 3. Results

**3.1 Bacteria Evaluation.** The data collected from the spread plates was reduced by selecting the most accurate plate count (typically between 30 and 300 CFUs). At a dilution factor of 10-6 the colony count was 152, meaning that the culture contained 1.52 CFU/μL. This concentration data was related to the OD results from dilutions of 100, 5×10-1, 10-1, 5×10-2, 10-2, 5×10-3, 10-3. The data was plotted and a regression line completed the relationship (Figure 3). With an R2 value of 0.992, the experiment had a strong fit. The following equation relates the OD of E. coli to the concentration of the suspension:

$$\text{Concentration} \left( \frac{\text{CFU}}{\mu\text{L}} \right) = 97261(\text{OD Measurement}) - 16109$$

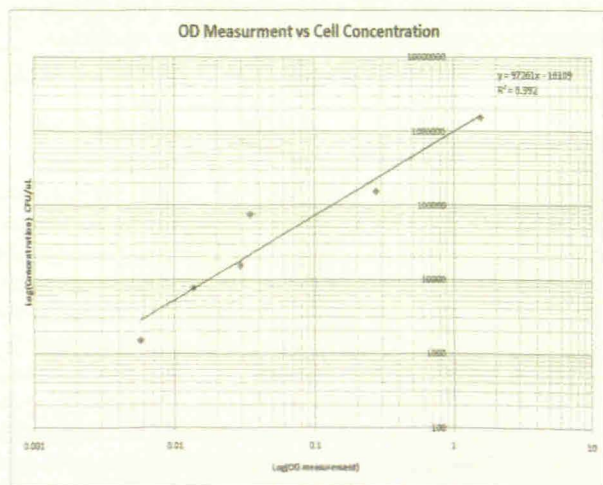


Figure 3: A standard linear regression graph relating OD and cell concentration in suspension

**3.2 Device Design.** The device worked as described in creating a channel for experiments to take place. The properties of MWNT clusters (magnetic susceptibility and bacteria adsorption) were utilized in the system.

**3.3 MWNT Concentration Determination.** After repeated injections, the ideal channel MWNT concentration was 4 mg/mL.

**3.4 Maximum Flow Rate Determination.** Flow rates of 2.0, 1.5, 1.0, 0.5, 0.2, and 0.1 mL/min were applied to a channel, which was saturated with MWNTs, for 1 minute and observed closely for MWNT cluster loss. On the basis of visual observations, 0.2 mL/min consistently had no loss of MWNT clusters. However, the 0.1 mL/min was selected for a safety factor of N=2. This prevents any MWNT loss during experimentation.

**3.5 Adsorption Capacity of MWNTs.** The experiments to determine the MWNT clusters' bacteria adsorption capacity were executed for varying cell concentrations in search for the threshold concentration, where MWNTs lose their ability to effectively filter E. coli, specifically the concentration where less than 95% are removed. The data below show important characteristics of the designed system. There was a strong correlation between the number of cells into the system and the % removal. Pearson's Correlation (r) relates two nonlinear parameters [13]. A Pearson's Correlation of 0.8 or above represents a good fit. In this case:

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{N}}{\sqrt{\left( \sum X^2 - \frac{(\sum X)^2}{N} \right) \left( \sum Y^2 - \frac{(\sum Y)^2}{N} \right)}} = -0.890$$

This represents a strong negative relationship. The variance in the system makes it difficult to quantify the system. As the graph shows, there are two distinct slopes (Figure 4). The intersection of the slopes will be defined as the capacity. Thus, the capacity for this system was estimated at 200,000 cells. The capacity per mg of MWNT was assessed on the basis of the volume of the system and the concentration of the MWNTs inside the channel:

$$\text{Capacity} = \frac{\text{Number of Cells}}{\text{Number of Carbon Nanotubes}} = \frac{[E. coli]}{[MWNT]}$$

$$\text{Capacity} = \frac{200,000 \text{ CFU}}{\frac{1.2 \text{ mL}}{4 \frac{\text{mgMWNT}}{\text{mL}}}} = 41667 \frac{\text{CFU}}{\text{mgMWNT}}$$

$$\text{Capacity} \cong 41,000 \frac{\text{CFU}}{\text{mgMWNT}}$$



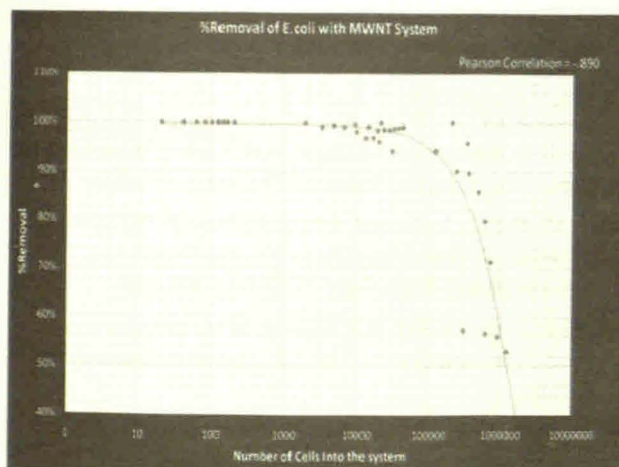


Figure 4: The percent removal of the MWNT system as a function of the number of cells injected

**3.6 Imaging.** Figures 5 to 7 show the results of the imaging experiment. Bacteria were infused into the system using the capacity testing procedure. MWNTs were isolated from the system and placed on a microscope slide and images were taken as described.



Figure 5: An image of MWNT alone taken with a light microscope at 40X optical zoom. Shows isolated MWNT clusters in solution

The images show that *E. coli* cells were adsorbed and retained by MWNT clusters. It appeared that the *E. coli* cells contacted MWNT clusters by diffusion or propelled by their own flagella mediated movement.

#### 4. Discussion and Conclusions

Some previous research using CNTs as bacterial filters [3] suggests that the bacterial filtration is based on size exclusion. In contrast, research conducted by Kim et al. [8] suggests that bacteria are spontaneously adsorbed on to the CNT clusters. The results of this study firmly support the findings by Kim's group, showing cells attached to all surfaces of MWNT clusters. Also, a size exclusion filter would be either 100% effective or 0% effective; however, the filter in this study had varying effectiveness based on the input concentration.

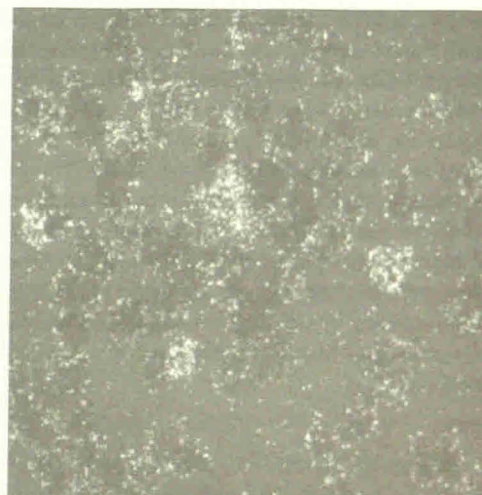


Figure 6: MWNT with epi-florescence microscopy (FITC filter) – showing live and dead cells as white dots. It is important to note that cells are highly concentrated around clusters of MWNTs



Figure 7: MWNT with epi-florescence microscopy (FITC and PI hybrid filters) showing live cells (blue-green) and dead cells (red). The cells are concentrated on the MWNT clusters. It appears that the number of dead bacteria outnumber the live. There is a MWNT cluster that shows live bacteria (circled)

Kim suggests that the specific chemistry, i.e., hydrophobic interactions and/or van der Waals interactions, between the MWNTs and the cell membranes may be responsible for the spontaneous cell adsorption [8].

The results from the study of the capacity experiment and imaging experiment show that the system was effectively filtering bacteria at 41,000 CFU/mg-MWNT. The two unique properties of the CNT clusters (i.e., bacteria adsorption and magnetic susceptibility) exploited in this study have proven a feasible system for the concentration of bacteria inside a micro-channel.

Further development of the CNT cluster-based process, however, is required through appropriate engineering designs

and optimizations to realize the unique advantages identified in this study. Future research may include redesigning the system using a Biological Micro-Electro-Mechanical Systems (Bio-MEMS) design, making a more efficient system that will maximize the capacity per quantity of MWNT. Future research may also quantify the toxicity of the MWNTs or test with other bacteria strains, viruses, and protozoa to determine their adsorption capacity. These would provide opportunities to extend the concepts demonstrated in this study to future applications, such as sampling units for environmental quality control or bio-sensing by offering ways to concentrate target biological agents, enabling us to accomplish the excellent properties of CNTs.

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Editor's note: Some of the figures in this study require full color imaging for maximum fidelity and accuracy. The black and white format of Inquiry reduces the quality of the images.

### Mentor Comments:

In his letter of support for Chris Nelson's article, Jin-Woo Kim clarifies the way in which this research adds to our knowledge of carbon nanotubes and their potential in future work:

*Mr. Chris Nelson's research with me involved a very exciting and challenging work to explore the unique properties of carbon nanotubes. The primary focus of the research was to study the possibility of developing an effective bacterial removal system by exploiting the unique properties of CNTs, in particular their high affinity to bacterial cells and magnetic property, which requires knowledge and tools of both biology and engineering. This cross-disciplinary research provided him an opportunity to strengthen his laboratory skills and broaden his knowledge in the fields of nanobiotechnology and biological engineering.*

*Chris has been very good at organizing his work. He also was an independent worker, requiring minimal supervision. While preparing a research proposal for the SILO Undergraduate Research Fellowship (SULF) a year ago, he excelled at working independently; however, he was not hesitant to ask questions when he had one. I was impressed by the level of the questions he asked regarding the research, indicative of his understanding of the topics of the research. As expected, he received the SURF grant. Furthermore, when getting started on the project, he not only sought to learn engineering and scientific concepts through literature search, but also sought out help to learn experimental techniques, including cell culturing, imaging, and analytical techniques. But then he just put his head down and practiced until he is very good at them. He has been very productive and also curious about the limits he believes the technology would undoubtedly face. In addition, he dealt with the frustrations of scientific experiments as well as writing with very mature equanimity while conducting the research and writing his thesis. He has not only successfully completed his research, but also he presented his*



*research results at the 2009 Annual Conference of Institute of Biological Engineering (IBE) on March 19 – 21, 2009. With my observation as his mentor for the last one and half years, I must say that I have found him to be a brilliant, very dedicated, and productive student.*

## Is Jove a Rock or a Leaner?

### Interpreting the Central Paintings of Pompeii's House of the Tragic Poet

By Rachel Newberry  
Classical Studies Program

Faculty Mentor: David C. Fredrick  
Classical Studies Program

#### Abstract

*When confronting a piece of ancient Roman artwork, the modern viewer faces the question, "How do we see like the Romans did?" Geographical, temporal, and cultural differences combine to make the process of understanding ancient art particularly complex. This piece attempts to bridge the gap between ancient Rome and the present through an analysis of the central mythological paintings located in regio VI, insula 8 of Pompeii. A three-dimensional model of the insula, created in the honors research colloquium "Digital Pompeii" at the University of Arkansas, is essential to the examination of the complex interaction between the paintings and their architectural context. By utilizing gender and gaze theory, examining Roman oratorical and memorization practices, and the rituals of daily life, this piece traces the social messages encoded in these paintings through the spatial sequence of the insula, paying particular attention to issues of gender instability, phallic power, and political hierarchy in Roman society.*

#### Introduction

For the contemporary viewer, understanding the frescoes that covered the walls of the *domus* of the ancient city of Pompeii presents a challenge. One might hail them as glorious and perhaps remark upon the talent (or, occasionally, the crudeness) of the artists who executed their designs throughout Roman domestic space. However, this does little to advance one's understanding of the various meanings encoded in the art and still less to illuminate the reception of the paintings by their original Roman viewers. As part of her critique of traditional approaches to art history, Griselda Pollock argues, "What we are taught is how to appreciate the greatness of the artist and the quality of art objects. This ideology is contested by the argument that we should be studying the totality of social relations which form the conditions of the production and consumption of objects designated in the process as art."

While Pollock aims to apply contemporary feminist discourse to 19th and 20th-century art history, this assertion certainly applies to the study of the domestic artwork of the ancient Pompeians, since "the quality and decoration of a Roman's house was closely linked with his social standing." Because domestic decoration was so closely tied to social hierarchy, applying gender theory, especially its analysis of power relations and phallicism, to one's study of Roman

decoration is useful – and arguably essential – to decoding its meaning.

I propose, then, a fresh interpretation of the paintings in the House of the Tragic Poet in Pompeii. My interest in the subject finds its genesis partially in my reaction to Bettina Bergmann's "The Roman House as Memory Theater," in which Bergmann discusses the experience of the paintings by their original viewers as linked to the Roman practice of oratory. My interest also derives from the creation of a three-dimensional model of regio VI, insula 8 in "Digital Pompeii," an honors colloquium class at the University of Arkansas.

The goal of this class was to reproduce Pompeii's artwork and artifacts in virtual reality, using drafting and video game software. By generating a 3-D searchable database through which I could navigate and view the artwork in its original context (on the walls), I found myself better able to visualize the interrelation between paintings and the ways in which their stories overlapped to create a complex narrative, one which commented on the sociopolitical situation and power structure of ancient Rome. By assuming the role of spectator, a role difficult to adopt simply by looking at a 2-D photograph of the decoration, I better understood how the Roman viewer would have interacted with the artwork. By utilizing gender and gaze theory, along with the readings of Bergmann, and informed by my experience of "walking" within the 3-D model of the insula, this article presents a fresh analysis of the artwork.

#### Space and Ritual in the Roman House

In order to understand the decorative themes utilized in Roman domestic space, the viewer must recognize that "[t]he architecture of the Romans was, from first to last, an art of shaping space around ritual." These rituals not only denoted formal ceremonies or household culta, but also daily interactions that shaped Roman life. Moreover, "the home was a locus of public life. A public figure went home not so much to shield himself from the public gaze as to present himself to it in the best light." The rituals of daily life occurred predominantly in the home, and each space's size, position in the domus, and richness of decoration acted as a signpost to the Romans who entered it, indicating both a room's function and its owner's status. Thus, the architect was faced with the task of creating a space that served as a place to carry out rituals and also communicated its utility to visitors and inhabitants alike.

A helpful example in understanding daily ritual is that of the *salutatio*, the visits that the *clientela*, or dependents, made to their patron, the paterfamilias of the home. The *salutatio* was essential to the political fabric of Roman life. *Clientela* waited upon their patron in the hopes of securing his favor and thus benefitting, and the size and socioeconomic standing of a patron's *clientela* contributed to his political clout. Its importance is made manifest in the layout of the domus, since Roman builders and artists shaped the *fauces* (entryway from the street), *atrium* (the central hall onto which the *fauces* opened), and *tablinum* (the main reception space at the end of the atrium, in which the paterfamilias sat and received guests) around the *salutatio*. As the *clientela* entered the domus, a "sequence of architecturally framed planes conducted the client's gaze to the paterfamilias in the *tablinum*," guiding the guest's movement from entryway to his goal.

The 3-D model is particularly useful here, for it allows one to retrace the path of the visiting client, moving through from the *fauces*, through the atrium and its numerous paintings, to the *tablinum*. The position of the patriarch, stationary in the *tablinum*, gazing out through the atrium and toward the *fauces*, can also be visualized (Fig. 2). The *fauces*-atrium-*tablinum* axis, then, served as a spatial guide that informed the visitor's movement through the domus, and the decorative schema it contained was instrumental in conveying the social rank and political importance of the paterfamilias.

This hierarchy of decoration existed throughout the entire house, coding each room's importance. Wallace-Hadrill suggests that one may rank Roman domestic spaces by placing them within the axes of public versus private and grand versus humble. The first of these categories delineates the difference between public rooms like the atrium and *tablinum*, to which all guests were permitted access, and private rooms into which only a select few were invited, such as the *triclinium* (dining room) or *cubiculum* (bedroom). The second axis refers to the social and political importance of both the spaces and their typical occupants, such that the rooms of an influential paterfamilias rank above those of a slave. Wallace-Hadrill combines the two modes of evaluation in order to situate each space in a given house into a social hierarchy.

Those rooms that occupied the elite end of the spectrum were hallmarked by a central painting containing mythological images to which "extraordinary prestige [was] attached." In contrast, lower-ranking spaces might be decorated with pastoral images, various animals, or still-life scenes. Thus, "heroic and divine scenes enjoy prestige in the same way as heroic poetry and tragedy do compared to the 'humble' genres of lyric or epigram." Based on its grandeur, the quality of the artwork (including fineness of detail and cost of the pigment used), and its subject matter, the content of the central images in a space communicated both the societal significance of their owner and the position the viewer occupied within it:

From the point of view of the ancient consumer...

decoration allowed a social orientation of two types: first,

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it helped to steer them within a house, guiding them round the internal hierarchies of social space...and second, it offered social orientation by contrasting one house with another, indicating the level of resources and social aspirations of the household.

Central mythological panels lie at the top of the decorative hierarchy and are among the most important clues to the relative status of the spaces in the domus. Their analysis, therefore, is essential to understanding the function of Roman wall painting; that is, how and what it meant in its original context.

### Contemporary Gender Theory and Roman Art History

The following analysis will assess the significance of the mythological panels from a domus in region VI, insula 8: the House of the Tragic Poet. I will take into account John Berger's work on gender relations and his description of the active male and passive female in artwork. I will also utilize Laura Mulvey's work on film theory, in particular her analysis of the viewer's scopophilic and sadistic gazes and what these indicate about gender relations. Carol Clover's evaluation of assaultive and reactive gazes in horror films is also pertinent. Susan Bordo's analysis of portrayals of the male body and what these views indicate about the gendered political and social hierarchy that produced this artwork will also be useful in my analysis.

Bettina Bergmann discusses "memory theater" and highlights the importance of understanding Roman decoration based on interrelations of paintings, as connected to the educated Roman's mode of mentally moving through a house. Her work provides insight into the ways in which these paintings were originally interpreted. Here, in particular, my use of the 3-D model of the insula will be vital, for rather than being forced to rely on a 2-D map detailing the placement of paintings in relation to one another, I "walked" through the domus and viewed the paintings from multiple angles. Finally, I will examine David Fredrick's analysis of erotic content of Pompeian paintings, based partly on Mulvey's concepts, and its gendered implications for Roman political and social structure. In particular, I am interested in analyzing phallic power and the potential for male exposure and vulnerability in Pompeian artwork to challenge and destabilize such power. Early gaze theory (like that of Berger) fails to take into account for these issues, but they do arise in later work, including that of Clover and Bordo.

According to Berger, a man's social presence depends upon the (phallic) power that he may potentially wield. In contrast, a woman's presence deals with her self-image and forces her to watch herself continuously, anticipating Foucault's concept of self-surveillance. This construct, when applied to artwork, figures the man as surveyor and the woman as the one surveyed. Thus, according to Berger, "men act and women appear. Men look at women. Women watch themselves being looked at."<sup>xiv</sup> As a result, women are objectified by the men who wield power over them.

Mulvey's analysis of the two gazes of the film spectator is also useful for the interpretation of the erotic scenes in Roman painting. Fetishistic scopophilia, a pleasure in looking at an idealized, fragmented woman, ignores her lack of penis, allowing the reader to deny the possibility that he, too, has the potential for suffering such a lack. Sadistic voyeurism, on the other hand, recognizes the woman's lack and watches as she is either forgiven or punished for it. The gazes tie in with Berger's point that often in artwork, men act upon passive, objectified women. This gendered power structure is vital to understanding the social and political hierarchy established in the Pompeian frescoes, and there is ancient precedent for its critical application, suggesting that this structure is not particular to modern media, but rather has ancient precedents.

Clover's work on gender in horror films deals with a twinned gaze, one of which invests its wielder with authority, while the other strips the gazer of power. The assaultive gaze is that of the predator, "a phallic gaze," that seeks to harm its victim; the reactive gaze is that of the victim or of the spectator seeking "to be oneself assaulted" by viewing "surrogates for one's own past victimized self."<sup>xvi</sup> In the case of the spectator, "Assaultive and reactive gazing are...housed in one and the same person," and thus one may identify in turn with both the assaulter and the assaulted. As Clover demonstrates, the role of the viewer and the sexualized rendering of the film's characters (or in our case, the painting's) should be taken into account in the work's analysis based on the information it provided about gender roles and "phallic cruelty."<sup>xvii</sup>

Clover's concepts have already found some application to ancient art. In "The Torturer's Apprentice," Helen Morales adapted Clover's gazes for her analysis of Parrhasius's art, based on Seneca's account in the *Controversiae* of the artist's practice of torturing a slave in order to portray accurately the body in pain. Morales argues that ancient Romans recognized the viewer's function in understanding decoration: "Seneca the Elder's *Controversiae*...is an important document which negotiates the social and moral responsibilities of the artist and the spectator...It considers the spectator's role as a victim of Parrhasius' art (the reactive gaze) and as an active consumer (the assaultive gaze)."<sup>xviii</sup> Morales's work, then, supports the application of modern gaze theory to ancient Roman artwork in order to better understand the messages encoded in the paintings.

Bergmann's methodology centers on the notion that "memory played a vital role in the creation and reception of Roman pictorial ensembles in domestic situations."<sup>xix</sup> As such, moving through the space and analyzing its artwork "constituted a process of remembering" that required the viewer to evaluate works in conjunction with other pieces throughout the domus.<sup>xx</sup> This process of remembering is closely linked with the "systematic memory training" which formed the groundwork of the education of elite Roman males: Cicero, Quintilian, and the author of *Ad herennium* recommended that students train their minds by likening memory to a large

house with numerous rooms through which one could move. In this house, everything has its place, and "it is the spatial order of the storage that allows for retrieval."<sup>xxi</sup> Because they were trained in the art of remembering, the ancient viewers would have applied this model to their experience of artwork in a physical house, especially as it prompted an association between images that produced complex meaning.<sup>xxii</sup>

Therefore, by analyzing the paintings from different points of view, taking into account various spatial relationships and the myths they represent, one may discover multiple meanings in the works and attempt to recreate the associations that the Roman viewer would have formed. Using a navigable 3-D model of the domus, such as the one generated in the "Digital Pompeii" class, serves as perhaps the best way to appreciate the kinaesthetic experience of the house (per Bergmann's model). As already mentioned, the model casts the user into the role of a spectator walking through the house, thereby aligning one's experience with that of the ancient Roman. If one hopes to understand the paintings in their original context (that is, to recreate the Roman process of memory association), the ability to view them in the house is vital. If the Roman visualized the memory process as a house, then the modern viewer must have access to a 3-D model of the domus in order to fully grasp how Roman structured their thought processes, and, by extension, their reading of the artwork.

Fredrick, on the other hand, focuses on the eroticism, and often violence, of the scenes, traits which many critics ignore. Though some, such as Wallace-Hadrill, place space in the decorative hierarchy based on the type of literature (ranging from epic to epigram) it references, Fredrick points out that the content of these erotic paintings "is usually not 'heroic'." "Rather, their erotic and/or violent content requires the consideration of gender as a means for encoding power (or powerlessness)."<sup>xxiii</sup> He notes that eroticism is "intimately connected with larger political and social relations of power"; as such, scenes in which a male asserts his sexual power can be seen as celebratory of influence, whereas those in which a male is made passive or is symbolically castrated represent fear of loss of said power.<sup>xxiv</sup>

While the tools of gaze theory will be essential to my analysis of central mythological images, Bergmann's "memory theater" will provide the point of departure for this examination. Bergmann analyzes the House of the Tragic Poet, a house featuring the most and best-preserved set of images in insula 8. It should be noted that this domus is fairly modest in size, indicating that, though rich in decoration, it did not occupy a place at the most elite end of the social spectrum. In general, Bergmann's examination makes sense, as she interrelates the myths on several different layers in order to show the complex relationships that can be drawn between them. For example, Bergmann points out that the women in the various paintings in the atrium all inhabit liminal stages, about to cross a threshold into a new part of their lives, be that step marriage (Juno), enslavement or rape (Briseis; Amphitrite),

or elopement with a lover (Helen).<sup>xxxv</sup> She also touches on more formal considerations, such as the way in which Hera and Aphrodite are positioned on the west and east side (respectively) of the fauces (1) entrance to the atrium, creating a dynamic of chastity versus eroticism (Fig. 1).<sup>xxxvi</sup>

However, she does not pay adequate attention to the erotic aspects of the works, ones which carry interpretive weight and which should be significant to the connection of domestic decoration to social power. By incorporating Bergmann's memory association with Fredrick's connection between eroticism and political power, and Clover's and Morales's assaultive and reactive gazes, the viewer may gain a fuller understanding of the gendered political and social commentary that the art offered to the Roman eye. At the same time, the emphasis on the exposure and vulnerability of the male body in my analysis of these paintings is not found in Berger or Mulvey's work on gaze theory, although perhaps it is anticipated in Clover and Bordo.

#### Analysis of the Central Paintings of the Atrium Area

The atrium (3) features six central paintings,<sup>xxxvii</sup> two of which are barely discernible, even in the 19th-century lithograph. On the south wall, to the west side of the fauces, is placed a painting of the wedding of Jove and Juno (Fig. 2). In it, Juno is clothed and faces the viewer; Jove lounges half-nude, holding Juno's arm and drawing her toward him. His position and lack of clothing make him a potential object of Mulvey's fetishistic scopophilia, for though he seems to assert his phallic power by claiming his bride, his stance leaves him open to the viewer's gaze. It is important to note that neither Berger nor Mulvey addresses the possibility of male vulnerability, focusing instead on male gazer/actor and a female recipient of the gaze. Nonetheless, when an exposed male body is portrayed in a manner similar to that of the female bodies to which they call attention, it is reasonable to use Berger's view of objectification and Mulvey's gazes to assess the power construct that the image implies. In addition, since Clover and Bordo consider the possibility of male exposure and the implications of this construct, their work is also pertinent here. Bordo would classify Jove's position as that of a "leaner," one with a soft, rounded, even vulnerable body. Similarly, on the east side of the fauces, a portion of a painting featuring a nude Venus is located (Fig. 3). Bergmann notes that this may have been a panel of the *Judgment of Paris*, in which case Venus's nudity would act as a display inviting male judgment. As it stands, Venus' naked body faces the viewer, though she covers her genitalia with her robe; this certainly fits with fetishistic scopophilia. Not only is Venus idealized, but she hides from the male viewer her difference from him, allowing him to fantasize without anxiety about her lack. Both paintings invite the viewer to fantasize, yet neither Jove nor Venus fully relinquishes power: Jove still dominates his bride, and Venus manipulates Paris into selecting her as the most beautiful, ultimately leading to the events of the Trojan War.

The west wall of the atrium contains two paintings that are significantly eroded and therefore more difficult to evaluate.<sup>xxxix</sup> One features Poseidon kidnapping Amphitrite, a situation that showcases sadistic voyeurism (Fig. 4). The viewer watches as the nude, helpless Amphitrite is carried away against her will by powerful Poseidon, a near-rape Bergman fails to address, at least in terms of its violence. Amphitrite's lack is punished in that she is forced into a sexual situation she does not desire; if she had a penis, Poseidon would probably not be a threat to her. One point in the painting that deviates from this model is Eros taking Poseidon's trident (an overt symbol of phallic power) away from him, seeming to indicate that his lust has unmanned him in some way; in Roman sexual ideology, a male who was unable to control his sexual desires jeopardized his dominant social position.<sup>xxxix</sup> The painting farther down the wall, closer to the tablinum, features the wrath of Achilles, but the lithograph fails to capture the majority of the painting, making it difficult to analyze a potential gaze (Fig. 4).<sup>xxxix</sup> Both paintings feature powerful men exercising their own will, despite the wishes of another, indicating that a narrative parallel can be drawn between them, in accordance with Bergmann's analysis of rhetorical pairings between paintings, and as seen in the 3-D model of the house.<sup>xxxiv</sup>

The east wall also contains two paintings, the southernmost of which features Agamemnon's abduction of Briseis (Fig. 4). Unlike Amphitrite, Briseis is fully garbed, and though she shrinks from her captor, she does not invite the sadistic gaze. The men in the painting, Agamemnon and Patroclus, are the more likely recipients of a fetishistic gaze, for both are half-nude, displaying idealized bodies. Patroclus especially seems an object of the gaze, as he is oriented toward the viewer, though he watches Briseis. Therefore, though Agamemnon asserts his phallic power here by forcing Briseis to accompany him, the painting is complicated by its portrayal of the men. The other painting contains an image of Helen about to board a ship, presumably to run away from Sparta and Menelaos to Troy with Paris (Fig. 4). She, like Briseis, is fully clothed and seems hesitant to take her next step; a man (possibly Paris) guides her by the arm and seems to push her toward the gangplank. Thus, the male is active and the female, passive, but the contrast is not as great as in Poseidon's abduction of Amphitrite or Agamemnon's claiming of Briseis as his war prize. The viewer is left to question whether Helen's actions are consensual or forced; either way, neither Helen nor her male counterpart invite either of Mulvey's gazes. The wall's theme seems to be the action of men forcing (or coercing) women to accord with their wills, a theme that would plausibly underscore the political power of the owner of the house. However, this exercise of power does not exclude a concomitant objectification of the male body by the gaze of the viewer. Even as these powerful men bend women to their respective wills, the viewer can employ a fetishistic or assaultive gaze and at least partially objectify these "active" males.

Room 6A also features two central mythological paintings, both of which lend themselves to interpretation based on gender and gaze theory. The south wall contains a painting of Europa and Jove in the guise of a bull. Europa is the epitome of the passive female object of the fetishistic gaze (Fig. 5).<sup>xxxv</sup> She wanly holds on to the bull, and her clothing billows away from her body. She turns toward the viewer, such that one breast is exposed and a hint of her genitalia may be seen. Even the bull has turned to gaze at her, implying that the viewer will as well. The bull's size, ability to carry her off, and horns all reference phallic supremacy and the man's ability to act on the woman. As 6A extends off of the atrium, if one stands at an angle oriented southwest, this image can be seen through an atrium doorway that separates the paintings of the abductions of Briseis and Helen. The relationship between the three paintings was made particularly clear by navigating the 3-D model (Fig. 7). This image of Europa's abduction, then, bridges the two other images, connecting all three as scenes in which men force their will upon women (with the possible exception of Helen).

The painting on the west wall of 6A, one of Phrixus and Helle, is less clear (Fig. 6). The siblings flee their step-mother's murderous intentions, aided by their mother who sends the ram to carry them away. Phrixus' power, then, is not sufficient to extricate himself (and Helle) from their trouble; however, he stays atop the ram, whereas Helle falls into the ocean and drowns. In the painting, Phrixus is physically imposing when compared to the diminutive Helle; though both are nude, fabric covers Phrixus' genitalia, whereas the drowning Helle is exposed to the viewer, indicating she may be the object of a sadistic gaze. Phrixus' phallic power here is murky: he is superior to Helle, but when compared to Jove, he is found wanting. As this painting, too, can be seen when looking through the 6A doorway, Phrixus may serve as a foil for the powerful Poseidon, Achilles, and Agamemnon, reflecting the fact that power, in the Roman social hierarchy, was not an either-or binary. Instead, it fell on a continuum, as seen in Wallace-Hadrill's progression from servi (slaves) through freedmen and clients, to close friends (*familiares*) and finally the owner himself.

The tablinum (8) contains one central painting which tells the story of Alcestis and Admetus (Fig. 8). An old woman prophesies Admetus's impending death; Admetus leans toward the viewer, stunned and overwrought, while a calmer Alcestis reaches out and comforts him.<sup>xxxvi</sup> All of the characters are clothed, and none of them seem particularly sexualized, rendering the gazes an ineffectual means of assessment. However, phallic power still comes into play, for a woman is delivering dire news to Admetus, and the latter realizes that he lacks the power to circumvent its coming to pass. Though not portrayed in the painting, the viewer realizes that Admetus is being punished by Artemis for failing to make a proper sacrifice to her. Though Apollo intervenes, he cannot negate the punishment, but can only allow for the possibility that a substitute life may be given in his place. This indicates that

Artemis wields the phallic power here (at least in this stage of the myth), though she is female, and that no male character can dissuade her, symbolically castrating Admetus.

Here, the artist makes use of the "material body as site of political struggle," referencing Fredrick's assertion that some paintings encode a fear of loss of political power by depicting a man's being stripped of phallic power.<sup>xxxvii</sup> However, it should be noted that later in the myth, Hercules agrees to descend into the Underworld and wrestle Death in exchange for Alcestis, then restores her to Admetus; to an extent, then, a male reclaims phallic power at the end of the story. Still, this scene allows one to understand better the dynamic of the decoration for visiting clients. As they walked through the atrium, they were surrounded by images of powerful men imposing their wills upon others, paintings which remind them of the power and influence of their patron. Using the 3-D model of the domus to retrace this path allows the modern viewer to experience this dynamic. In the tablinum, however, a more cautionary tale exists, one that warns the male visitor against hubris, for should he fail to behave rightly and honorably, he could potentially lose social and political power. As such, the didactic nature of this painting contrasts with the exposed male bodies that the visitor encountered on his walk through the atrium.

### Analysis of the Paintings in the Peristyle Area

Another significant mythological painting is found in the peristyle (10), one detailing the sacrifice of Iphigenia (Fig. 9). Here the powerful role of Artemis continues. Iphigenia resembles Europa, in that she is nude (with the exception of her genitalia) and wholly exposed to the viewer, powerless against the sadistic and fetishistic gazes, as well as against her captors. According to Mulvey, the sadistic and fetishistic gazes are distinct and should not exist simultaneously in a scene; however, this image complicates Mulvey's reading because the portrait of Iphigenia does expose her to both. All of the men have averted their gazes from her: her father, Agamemnon, hides his face beneath his cloak, and the others look toward the sky, where Artemis presides. According to the myth, she will save Iphigenia from being killed. For now, however, she remains steadfast in her demand that Agamemnon sacrifice the girl to her in order to make reparation for killing her sacred deer, then boasting of his skill. He is powerless to refuse her, as are the other men in the scene; moreover, he has a larger political goal at stake (namely obtaining a favorable sailing wind for the Greeks, so that they can attack Troy). Thus, staying in the goddess's good favor is essential.

This continues the theme of the tablinum, in that a fear of powerlessness pervades the scene. The tablinum contains a large window that opens onto the peristyle and allows the spectator to view the painting of Iphigenia (as demonstrated by the 3-D model). It should be further noted that both paintings praise the sacrifice of women (those even more powerless than the suffering men) in order to save the men from further doom. This femicide could be read as an extreme fulfillment of

sadistic voyeurism, one not included by Mulvey but pertinent nonetheless. Iphigenia's death stands in contrast with that of Alcestis and highlights the dualism of the woman as willing or unwilling sacrifice. Whereas Agamemnon (and Artemis) chooses to kill his daughter (allowing her death to be seen as punishment and therefore sadistic), Alcestis voluntarily offers her life in exchange for her husband's (which could be read as either self-abnegation or a desire to exchange her physical life for social fame).

The triclinium (15) contains three central pieces; Bergmann does not address these paintings in her reading of the house, but they contribute to understanding the overall decorative implications of the House of the Tragic Poet. The painting on the north wall is identified in *Pompeii: pitture e mosaici* (an encyclopedia of all of the decoration found at the excavated sites of Pompeii, including photographs and descriptions) as the "Sale of Cupids."<sup>xxxviii</sup> Per the myth, Cupid is imprisoned as though he were a runaway slave, a punishment for the crimes he has committed against various lovers. His captors put him on sale, thus inverting his former position of power into that of slavery. In light of the myth, Cupid's body invites both a fetishistic and scopophilic gaze: the curve of his body (which figures him as a "leaner") and the exposure of his genitalia invite the spectator (who knows that Cupid will soon be punished) to gaze upon him. Just as the tale of Admetus serves to caution the viewer, so could this painting be read as a warning against the dangers of desire, which could enslave its victims like Cupid, should they allow it to dictate the course of their actions.

The painting on the south wall returns to Artemis, who stands with Callisto (Fig. 10). The painting has suffered serious damage, rendering it difficult to evaluate the precise condition of the figures. However, it seems that they are all clothed, and neither woman turns toward the viewer to invite his gaze. Phallic power permeates the myth, in that Jove rapes Callisto; however, he must assume the form of a woman (usually Artemis) in order to do so.<sup>xxxix</sup> This calls to mind Fredrick's point about the gender-bending present in Roman art and literature, as demonstrated by the frequency with which Hermaphroditus is depicted in painting and sculpture and the subordination of the poet-lover to the *puella* in elegy. Jove is unable to assert his phallic power outright, but rather must associate himself with femininity (typically the antithesis of power) in order to achieve his desires. This ties in with the image displayed in the peristyle (10), in which Artemis exerts power over Agamemnon. A persistent narrative of men being stripped of power, or at least having their power lessened, emerges and further connects with the image in the tablinum (8). The painting of Cupid, then, serves as a humorous foil to the reversal of power frequently experienced by "serious" male figures in many of the paintings. As the triclinium opened onto the peristyle, a visual connection between the two rooms existed, and the spectator would have linked the images they contained (as demonstrated by the 3-D model).

A clearer-cut case of male superiority appears on the room's east wall, which features Theseus abandoning Ariadne (Fig. 11). He stands on the ship and turns to gaze at the reclining Ariadne. (Since the painting is also damaged, it is difficult to determine whether she is sleeping or simply powerless to prevail upon him to stay.) Her position and her nudity invite both a scopophilic and a sadistic gaze (not unlike Iphigenia), for her lover abandons her, leaving her exposed and alone. The 3-D model shows the correlation between these two paintings, in that the viewer facing the painting of Theseus and Ariadne also experiences that of Iphigenia, which is positioned on the left, on the rear wall of the peristyle (Fig. 12). The informed viewer knows that soon Dionysus will encounter and rape Ariadne, furthering her punishment, though this is also regarded as a "wedding" and thus a kind of "reward" (from the man's perspective).

### Conclusions

While it is impossible to experience Roman artwork as the Romans did, the ability to navigate through a virtual, three-dimensional Pompeii allows one to reconstruct the erotic narrative that the paintings create. Locating oneself in the atrium of the House of the Tragic Poet, for example, and facing every angle possible is much more useful in understanding how the paintings reflect and speak to one another than is viewing them printed in a two-dimensional reproduction. It further allows one to retrace the path of the salutatio, assuming the position of the clients who walked through the fauces, into the atrium and toward the tablinum, all the while surrounded by central mythological images that told stories of power exercised and power lost. Conversely, one might stand in the tablinum, in the position of the *paterfamilias*, and look out across the atrium toward the entrance to the domus. Here one can view the decorations commissioned to hallmark one's social standing and political power and perhaps observe their effects on one's dependents (Fig. 2). Books and flat images, valuable though they are, cannot offer this experience, and for this reason, the virtual model is indispensable in understanding Pompeian art.

Moreover, the model aids in the application of techniques such as those suggested by Fredrick and Bergmann, both of which evaluate the meaning of artwork and of the implications of said meaning regarding Roman daily life. As Bergmann's work places emphasis on the rhetorical and compositional relations between the paintings, the models constructed through the Digital Pompeii Project are powerful aids in visualizing and critiquing Bergmann's assessment. As previously noted, Bergmann suggests that the process of remembering in Roman rhetorics can be enhanced by visualizing memory as the mind's movement through a domus containing many rooms. The details of a speech, in memory, become the decoration on the walls, and are thereby entwined with the paintings one encounters. The process of ordering the scenes, interrelating them, and drawing conclusions is equated with the ability to synthesize knowledge into a verbal argument. Thus,

Bergmann demonstrates that the lucid rhetorical arrangement of “paintings” exhibits the Roman male’s oratorical skills, political power, and social position – his phallic power, in other words. The 3-D model allows the modern viewer to move through a Roman domus, providing insight both into the process of reading the paintings and that of ordering the Roman mind.

This visualization, then, serves to organize the orator’s thoughts by superimposing the different portions of his speech over the artwork in his mental domus. It might be said that the paintings he sees comprise an inner narrative. Through its content, the artwork of regio VI, insula 8 allows this progression to be evaluated, in light of Mulvey’s work, as an erotically charged narrative that prompts scopophilia and voyeurism on the part of the viewer and invites movement between gendered positions. It seems as though this eroticism would undermine the task at hand, namely the organization of a political speech. After all, mental images of a nude Venus manipulating Paris or of a glorious Artemis commanding Agamemnon do not appear conducive to composing and delivering a speech on the often weighty topics of Roman public oratory: the management of the grain supply, for instance, or a military campaign in Asia Minor. Visualizing sexually-charged, role-reversing scenes would potentially destabilize the oratorical narrative, based on their ability to distract, confuse, or even frighten the orator. The use of images that challenge established gender roles in this memory theater questions the traditional view of Roman power and oratorical prowess as aggressively masculine.

Corbeill’s work on the importance of movement to the Roman orator supports the assertion that gender-bending occurred frequently in the political arena. Cicero writes about the proper set of bodily movements, or *habitus*, to which an orator should confine himself, rejecting those that he deems either effeminate or rustic. To act outside of the *habitus*, in his view, reflects upon the character of the actor, to the point of implying that a man is “sexually submissive,” and thereby lacking in phallic power or political influence. Nevertheless, notable politicians, including Julius Caesar, rejected the *habitus* of the dominant political party in order to set themselves apart as opposing this group’s political stance. In fact, Caesar went so far as to don “nonmasculine dress” as a means of “self-advertising” his opposition to Sulla’s policies. The salient point to be made is that Caesar and other “popular” orators engaged in gender-bending behavior not to establish a sexual difference, but rather a political identity apart from their contemporaries.

Hadrian’s immortalization of his lover Antinous’s image throughout the Roman Empire provides an additional example of how hegemonic gender roles could be manipulated to political ends.<sup>xliii</sup> Caroline Vout suggests that Hadrian’s decision to reproduce Antinous’s visage on marble busts, coins, memorials, and inscriptions was not motivated solely by the emperor’s desire to deify his deceased lover. Rather, Hadrian intended to force his subjects to think of their ruler every time

they encountered a visual representation of Antinous, thereby insinuating himself in Roman daily life. His method of doing so, however, entailed challenging the sexual relationships deemed acceptable for a “real man,” or *uir*, which did not include “his sleeping with a freeborn citizen.”<sup>xliiv</sup> Despite this violation of social mores, representations of Antinous were not automatically fetishized and stripped of phallic power. Vout argues that Antinous remained a “young intact male” (rather than being associated with a male slave or being deemed effeminate) and therefore cannot be read according to Mulvey’s gaze theory, because he concomitantly possessed power and was rendered a passive, objectified body by spectators.<sup>xliv</sup>

Based on my analysis of Roman decoration and on the aforementioned examples, I suggest that one would be mistaken in figuring the political and social power structure of the Roman Empire as solely based on phallic power and aggressive masculinity. Gender-bending not only occurred, but was also successfully utilized as a tool in the political machinations of various orators. Occasionally, conflicts were even resolved via the utilization of traditionally-feminine traits, rather than masculine strength or combat. Suetonius records that a certain Gallic chief was so affected by the beauty and softness of Augustus’s body that he eschewed pushing the emperor off a cliff, as he had previously planned. Thus the qualities that saved Augustus’s life were antipodal to the masculine ideal.<sup>xlii</sup> The erotically-charged encounter imbued Augustus with a certain power, even if it did deviate from the Roman meme of proper masculinity. This, too, speaks to the need to reconsider notions of Roman masculinity and phallic power in order to account for deviations from these paradigms. The artwork of Pompeii supports this complication and aligns itself with a reading that takes into account the importance of gender-bending in obtaining political power. Therefore, when we view and move past the paintings in the House of the Tragic Poet, we see the reflections of a much more flexible and unstable “phallic power” than we might expect, but one which is consistent with the complex gender positioning of elite oratory.

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- <sup>xvii</sup> Clover, 175.
- <sup>xviii</sup> Helen Morales. "The Torturer's Apprentice: Parrhasius and the Limits of Art." *Art and Text in Roman Culture*. Ed. Jaś Elsner. Cambridge: Cambridge University Press, 1996, 184.
- <sup>xix</sup> Bergmann, 225.
- <sup>xx</sup> Bergmann, 237.
- <sup>xxi</sup> Bergmann, 225.
- <sup>xxii</sup> Bergmann, 226.
- <sup>xxiii</sup> David Fredrick. "Beyond the Atrium to Ariadne." *Classical Antiquity*. 14.2 (1995), 267.
- <sup>xxiv</sup> Fredrick, 268.
- <sup>xxv</sup> Bergmann, 245.
- <sup>xxvi</sup> Bergmann, 245.
- <sup>xxvii</sup> For the location of the paintings as situated in the House of the Tragic Poet, see Fig. 2.
- <sup>xxviii</sup> These 19th century lithographs by Pedicini (contained in *Pompei: pitture e mosaici*) are valuable in analyzing the atrium's artwork, as some of the paintings are no longer extant, and minimal photographic evidence of their contents remains. Bergmann also relies upon the tempura paintings of Raoul-Rochette to decipher the room's artwork (233).
- <sup>xxix</sup> Susan Bordo. *The Male Body*. New York: Farrar, Straus, and Giroux, 1999, 186.
- <sup>xxx</sup> Bergmann, 237.
- <sup>xxxi</sup> Ibid.
- <sup>xxxii</sup> Jonathan Walters. "Invading the Roman Body." *Roman Sexualities*. Eds. Hallett and Skinner. Princeton: Princeton University Press, 1997, 29-43.
- <sup>xxxiii</sup> As Bergmann points out, the painting may be identified based on the stance of the sandaled foot and the red cloth garbing the figure (237).
- <sup>xxxiv</sup> Bergmann, 226.
- <sup>xxxv</sup> While Bergmann mentions this painting, she pays minimal attention to it in the course of her interpretation. She does <https://scholarworks.uark.edu/inquiry/vol10/iss1/1>

describe the visual effect of looking into room 6A from the atrium, however (251-55).

<sup>xxxvi</sup> A key text for interpreting this painting is Euripides's play *Alcestris*, as noted by Bergmann (pp 232-34).

<sup>xxxvii</sup> Susan Bordo. *Unbearable Weight: Feminism, Western Culture, and the Body*. Berkeley: University of California Press, 1993, 7. Also, Fredrick, 268.

#### Mentor Comments:

David Fredrick provides background information about the use of a 3D searchable database of Pompei, explaining how Rachel Newberry used this tool to explore interpretations of the paintings in the House of the Tragic Poet. He writes:

*I am delighted to write a letter in support of Rachel Newberry's publication in Inquiry. Rachel's piece had its origin in an undergraduate research colloquium, "Digital Pompeii," that aims to produce a 3D searchable database of wall painting, mosaics, and artifacts from Pompeii. Its core is a 3D model of Pompeii, linked to a database developed from Pompei: pitture e mosaici (PPM), a multivolume Italian encyclopedia of wall paintings and mosaics. The 3D model is published through a game engine, so that it can be navigated online, with data about paintings and mosaics pulled from the database into the web player. The course is limited to 6 students each semester, and each student is responsible for one insula (city block) from Pompeii--they trace the 2D plan of the insula, extrude it into 3D, scan the images the artwork from PPM, and input data on the artwork into a database. They also read secondary works on the history of Pompeii, the development of the Roman house, and interpretation of decorative ensembles; the final paper for the course consists of a critique of current interpretive approaches to Pompeian art based on what they have learned modeling their insulas in 3D, and being able to walk through their spaces in virtual space.*

*Rachel's article evolved from her final paper for the course, and it certainly illustrates the power of the 3D database to further research on Pompeian art. Her piece considers the most important published interpretation of the House of the Tragic Poet in Pompeii (VI.8.3-5), "The Roman House as Memory Theater" by Bettina Bergmann, and notes that while it draws important connections between the arrangement of the paintings and Roman oratory, it neglects the erotic content of many of the paintings and the relation of this to issues of gender and power. The piece argues that this content is particularly striking to a viewer moving through the House of the Tragic Poet as reconstructed in virtual space--since most of the paintings have faded or been removed to the Naples Museum, the original decorative program cannot be appreciated by visiting the actual house today. In exploring the connection between eroticism and power, Rachel makes sophisticated use of gaze theory; drawing on film criticism and contemporary art history; however, she notes that the paintings in her insula challenge the clear division between male activity and female objectification found in much gaze*

theory. Rather, the male body is frequently exposed, made vulnerable as the object of the viewer's gaze. She concludes the piece by pointing that the display of gender-bending and male vulnerability in the paintings stands in pointed contrast to the emphasis on the assertion of elite male status through oratory found in Bergmann's interpretation. She argues for a more complex and nuanced view of Roman masculinity, noting that gender instability seems to have been an effective part of self-presentation for politicians in the late republic, and that "passive" eroticism remains part of the public persona of many Roman emperors.

In its combination of 3D content, art history, and gaze theory, this is a remarkable piece of work, combining critical theory with an eye for how the paintings actually work in their (virtual) space. While it emerged from the larger research aims of "Digital Pompeii," this submission certainly stands independently, both for its analysis of the particular paintings found in the House of the Tragic Poet, and for its suggestion of how eroticized male bodies might be understood in the context of masculine display and social competition in Roman culture. In this case, the student has essentially created the tool (the 3D database) that pushed the interpretation along--a tool that is not currently available to any other researchers on Roman art. This involved learning a whole new set of skills (in Cinema 4D, Photoshop, and the game engine Unity) which again are very rare among art historians doing research on Pompeii. Further, Rachel drew upon film theory to inform her approach to erotic content; this is not easy reading, and she elegantly summarizes some quite difficult concepts, showing clearly how they are at play in the Roman paintings. This submission is the result of extremely hard work acquiring software skills, sifting and entering data from PPM, working through secondary sources on Roman art and Pompeii, and considering how contemporary gaze theory could illuminate these paintings, and contribute to our understanding of how Roman identity is defined (and sometimes challenged) in domestic decoration. Rachel's work is worthy of publication in a national journal. Its publication in *Inquiry* represents the very fine work that our students do in the humanities at the University of Arkansas.

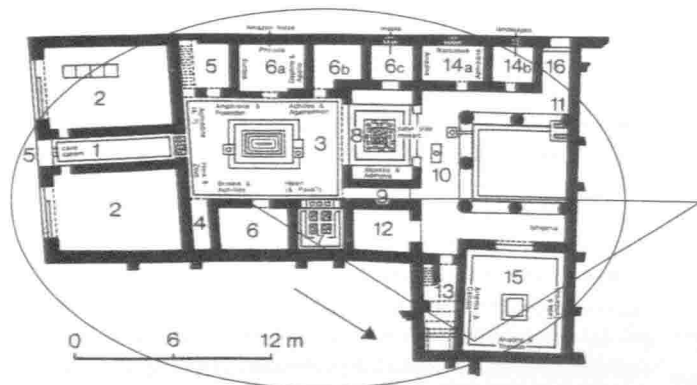


Figure 1. Map of the location of the artwork in the House of the Tragic Poet. Bergmann, "The Roman House as Memory Theater." *The Art Bulletin*, 76.2 (1994), 250.

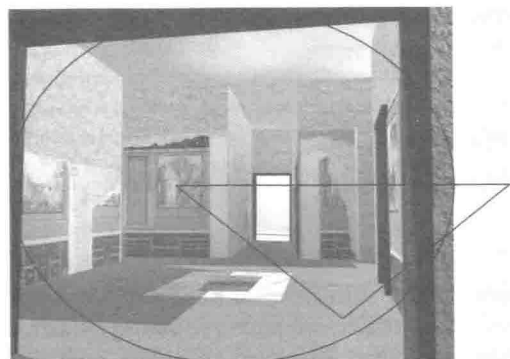


Figure 2. South wall of atrium (3), House of the Tragic Poet. *Wedding of love & Iuno* (left) and fragment of *Venus*. Render from 3-D model.

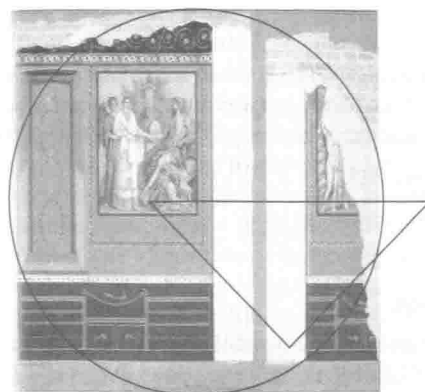


Figure 3. *Wedding of love & Iuno* and Fragment of *Venus*. South wall of atrium (3), House of the Tragic Poet. Pompei: pitture e mosaici (1990) Vol. IV, 539.

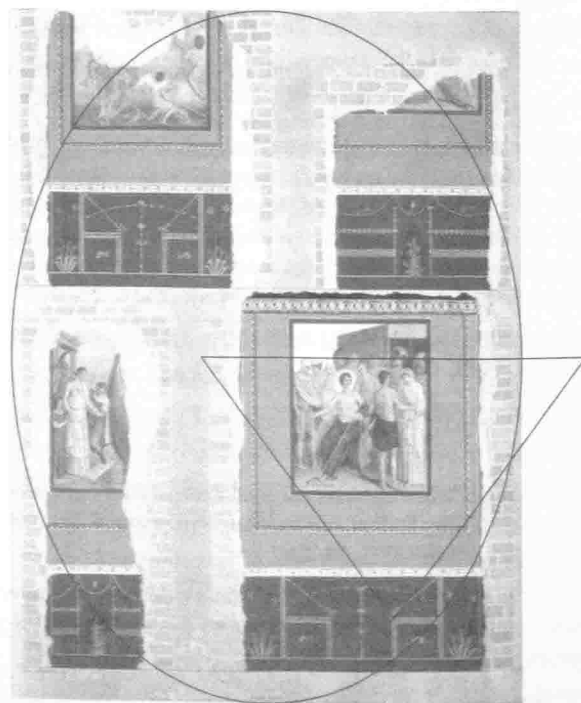


Figure 4. Lithograph of *Poetikon Kidnapping/Abduction: The Wrath of Achilles: Helen and Paris?* and *Agamemnon Abducting Briseis*. West & east walls of atrium (3), House of the Tragic Poet. AOS 263 (For. Pedicini)



Figure 5. Europa and the Bull. Cubiculum (6A), House of the Tragic Poet. Pompei: *pittura e mosaici* (1990) Vol. IV, 595.



Figure 6. Phrixus & Helle. West wall of cubiculum (6A), House of the Tragic Poet. Pompei: *pittura e mosaici* (1990) Vol. IV, 597.

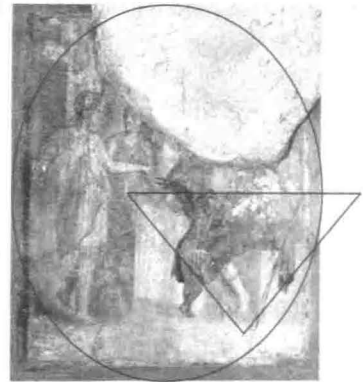


Figure 10. Artemis & Callisto. South wall of triclinium (15), House of the Tragic Poet. Pompei: *pittura e mosaici* (1990) Vol. IV, 581.



Figure 7. Atrium (3) with image from 6A, House of the Tragic Poet. Render from 3-D model.

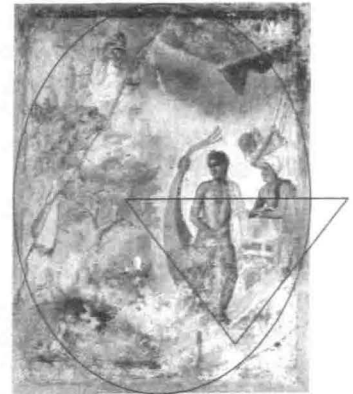


Figure 11. Theseus Abandoning Ariadne. East wall of triclinium (15), House of the Tragic Poet. Pompei: *pittura e mosaici* (1990) Vol. IV, 576.

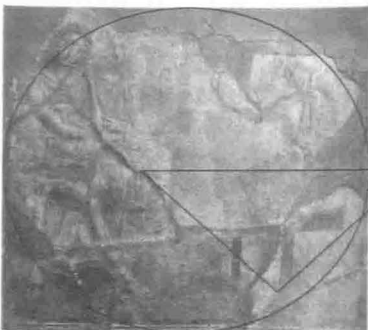


Figure 8. Alcestis & Admetus. Tablinum (8), House of the Tragic Poet. Pompei: *pittura e*



Figure 9. Sacrifice of Iphigenia. Peristyle (10), House of the Tragic Poet. Pompei: *pittura e mosaici* (1990) Vol. IV, 552.

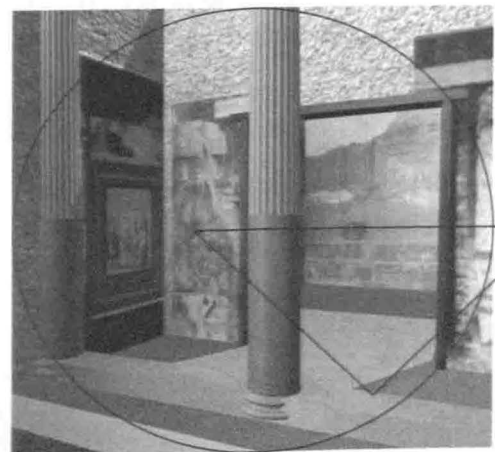


Figure 12. Sacrifice of Iphigenia and Theseus Abandoning Ariadne. Peristyle (10) & triclinium (15). Render from 3-D model.

## ACCOUNTING CONSERVATISM IN INTERNATIONAL FINANCIAL REPORTING STANDARDS AND U.S. GENERALLY ACCEPTED ACCOUNTING PRINCIPLES

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### Abstract

*During the past five years, the number of U.S. citizens who own foreign securities has increased by thirty percent. This trend has led to the need for a uniform accounting system that would increase the comparability and consistency of financial statements across countries in the world. Today, over 100 countries have adopted International Financial Reporting Standards (IFRS) as their primary accounting system. The European Union required the use of IFRS in 2005. In the U.S., the Securities and Exchange Commission is considering the adoption of IFRS in 2014.*

*IFRS and U.S. Generally Accepted Accounting Principles (GAAP) are different in many ways. U.S. GAAP is more detailed, with strict rules and guidelines to follow. In contrast, IFRS allows more room for accountants to make judgments in preparing financial statements and auditing them. This has led to the assumption that IFRS would open the door to earnings management and decrease the conservatism of financial statements. Conservatism is "the accountant's tendency to require a higher degree of verification to recognize good news as gains than to recognize bad news as losses" (Basu, 1997). Conservatism helps prevent managers from manipulating income and earnings per share (EPS). While there are many studies on accounting conservatism in U.S. GAAP, few or no studies have been done to determine the impact of conservatism in IFRS. This study was conducted to determine whether IFRS is more conservative than U.S. GAAP by comparing the book-to-market value (BTM) between IFRS firms and U.S. GAAP firms. Lower BTM values are associated with greater firm conservatism.*

### 1. Introduction

During the past few years, the number of U.S. citizens investing funds in foreign companies has increased dramatically. According to the Securities and Exchange Commission (SEC), two-thirds of U.S. investors own foreign securities, a thirty percent increase in the past five years. This rising trend of investing in foreign companies has created the need for a uniform accounting system that would increase the comparability and consistency of financial statements across countries. Today, over 100 countries have adopted International Financial Reporting Standards (IFRS) as their primary accounting system. In Europe, the European Union (EU) has required "companies incorporated in one of its Member States and whose securities are listed on an EU regulated market

to use IFRS beginning with their 2005 financial year" (SEC Release 33-879).

On February 18, 2000, the SEC issued a Concept Release "seeking input on convergence to a high quality global financial reporting framework while upholding the quality of financial reporting domestically" (SEC Releases 33-7801). On September 18, 2002, the SEC formally committed to the convergence of U.S. GAAP and IFRS in the Norwalk Agreement. Almost five years later, on July 3, 2007, the SEC issued for public comment a proposal that would allow foreign issuers to file financial statements according to IFRS standards without having to reconcile these statements to U.S. GAAP (SEC Release 2007-128).

On November 15, 2007, the SEC enacted a rule amendment that eliminated the convergence from IFRS to U.S. GAAP for all foreign issuers, with an implementation date of May 4, 2008 (SEC Releases 33-8879). This rule helps promote investments in non-U.S. companies, because foreign companies would no longer need to spend money converting their financial statements from IFRS to U.S. GAAP. The SEC also believes that this rule would "help American investors better analyze and get more readily comparable financial information from the U.S.-registered foreign companies in which they invest." In fact, former SEC Chairman Christopher Cox states that, "Consistent application of international accounting standards will help the two-thirds of U.S. investors who own foreign securities to understand and draw better comparisons among investment options than they could with a multiplicity of national accounting standards" (Press Release 2007-235). In his statement on October 24, 2007, Robert Herz, Chairman of the Financial Accounting Standards Board (FASB), suggested that the U.S. should set specific timelines to accommodate any changes necessary to support a move to IFRS, including training to potential users of financial statements.

On August 27, 2008, the SEC proposed a "Roadmap" that could lead to the adoption of IFRS in the U.S. in 2014 (SEC Releases 2008-184). The SEC would decide in 2011 whether adopting IFRS would be beneficial to investors and the public interest. Currently, there are opposing opinions regarding the adoption of IFRS. For example, in a 2009 survey by Deloitte & Touche LLP, one of the "Big Four" public accounting firms, 75% of the respondents favored a movement toward a uniform global accounting standard, such as IFRS. Paul Volker, former chairman of the International Accounting Standards

Committee Foundation and current chairman of President Obama's Economic Recovery Advisory Board, states that "I do think we ought to be working toward international accounting standards and have them become the standard around the world under the general aegis of the International Accounting Standards Board (IASB), and there's been a lot of progress in that direction." Others, such as SEC Chairman Mary Schapiro, are more skeptical about the movement of IFRS adoption in the U.S. Specifically, Schapiro is concerned about "the pace of the timeline, the independence of IASB, the quality of the standards themselves, and the cost companies must incur in making the conversion" (CFO). The SEC estimates that each firm would have to invest \$32 million in adoption of IFRS, which might be a major barrier to smaller firms.

The possible convergence of U.S. GAAP to IFRS in future years would have a major impact on the accounting profession. Many questions have not yet been addressed about the implications of this convergence. Currently, there has been little or no research on the differences between conservatism in U.S. GAAP and conservatism in IFRS. Conservatism is an important topic in accounting. It involves the need for higher verification to recognize gains than to recognize losses. In ambiguous circumstances, conservatism will understate net income (Basu, 1997). This means that investors will be given information where they are receiving the most conservative number for net income, which hopefully leads to better investment decisions than if they are provided an overstated net income number.

This topic of conservatism in IFRS compared to conservatism in U.S. GAAP is addressed in this paper. BTM value (book value of equity divided by market value of equity) is one of the most important factors in determining conservatism. This paper compares BTM values between IFRS firms and U.S. GAAP firms to determine which accounting system is more conservative. Other factors, such as total assets and the skewness of total assets, are also controlled in the analysis. Before framing the research questions of this study, additional background information is provided.

## 2. Major differences between IFRS and U.S. GAAP

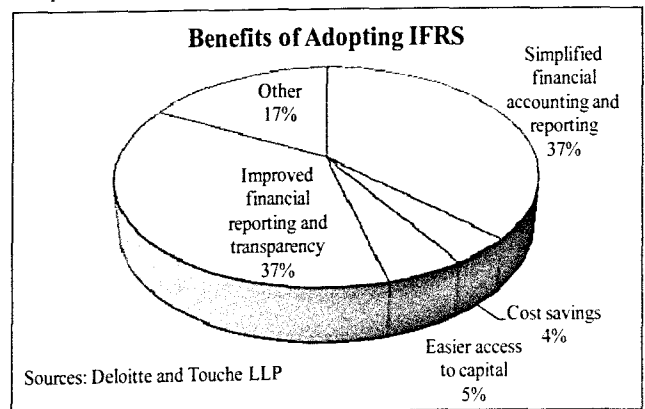
A key difference between IFRS and U.S. GAAP is that IFRS tends to be principles-based while U.S. GAAP tends to be rules-based. A rules-based accounting system is more detailed, with specific rules and guidelines to address as many unforeseen circumstances as possible. In contrast, a principles-based accounting system provides a more "conceptual basis for accountants to follow instead of a list of rules" (The CPA Journal Online). As a result, a principles-based accounting is more flexible, and allows more room for accountants to make choices.

Both accounting systems have their own advantages and disadvantages. The rules-based accounting system such as U.S. GAAP is normally criticized for its complexity and inflexibility. For example, in the article "Defining Principles-Based Accounting Standards," Shortridge and Myring state

that the rules-based accounting system "has made standards longer and more complex, and has led to arbitrary criteria for accounting treatments that allow companies to structure transactions to circumvent unfavorable reporting. In addition, the quest for bright-line accounting rules has shifted the goal of professional judgment from consideration of the best accounting treatment to concern for parsing the letter of the rule." Compared to a principles-based accounting system such as IFRS, the U.S. GAAP guidelines are much longer and more complex, with 25,000 pages of rules and standards compared to 2,500 pages of IFRS, according to PricewaterhouseCoopers, one of the "Big Four" public accounting firms. In fact, when talking about the complexity of GAAP, Robert Herz, FASB Chairman, said, "We've got something that's suited to a different era, that's not global. I believe it's better to create something new than to patch up something old and outdated."

In 2008, Deloitte & Touche LLP surveyed 200 finance professionals and found out 42% of the respondents indicated that their companies would prefer the earlier adoption of IFRS if permitted. Thirty seven percent of those respondents who favored the earlier adoption of IFRS thought the simplicity of IFRS was one of the major benefits of this accounting system. Graph 1 below demonstrates the proportions of benefits from adopting IFRS.

Graph 1.



One drawback of U.S. GAAP would be eliminated by the use of IFRS, because IFRS is frequently praised for its simplicity and flexibility. However, IFRS flexibility is also a disadvantage. For instance, even after the U.S. adopts IFRS, the financial statements between companies in the same industry may not be comparable, because IFRS allows more room than GAAP for accountants to make judgments in preparing financial statements and auditing them. This can open the door to earning management, where managers manipulate income to increase a firm's net income and earnings per share (EPS). Commenting on the flexibility of IFRS, Financial Accounting Standards Board (FASB) Chairman Robert Herz stated, "Basically you can do almost anything you want."

This can also lead to large differences in earnings reporting. A study by Jack T. Ciesielski, the publisher of The

Analyst's Accounting Observer, found that, "among the 137 companies reporting 2006 results under both GAAP and IFRS, 63% showed higher earnings with the international standards. For the median company, profits jumped by 11%" (BusinessWeek). Further, many countries that adopt IFRS add their own exceptions, making the international accounting standard not so global after all. Lawrence A. Cunningham, a law professor at George Washington University, said, "We may get something that people think uniform but is not. There is a real risk of a veneer of comparability that hides a lot of differences." In their 2008 survey, Deloitte & Touche LLP found 33% of those respondents who favored the adoption of IFRS cited the "lack of accounting technical guidance" as a major challenge of IFRS. Graph 2 illustrates the proportions of major challenges of adopting IFRS.

Graph 2.

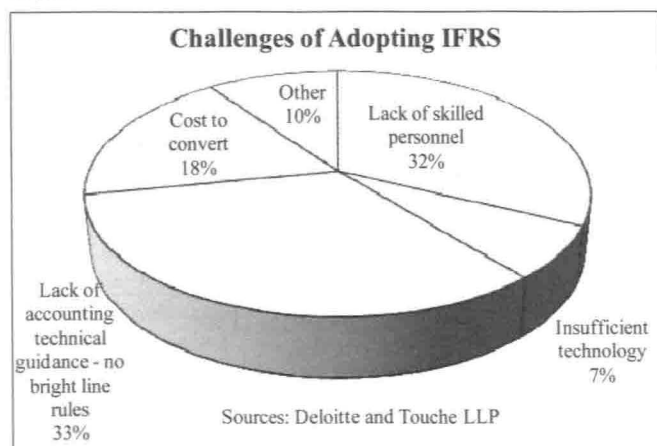


Table 1 demonstrates some of the major differences between IFRS and U.S. GAAP, and shows how strictly rules-oriented U.S. GAAP is compared to IFRS. The table is adapted from "IFRS and US GAAP – A Pocket Comparison" by Deloitte & Touche LLP.

Table 1.

Topic	IFRS	U.S. GAAP
Reporting a separate line item for "total comprehensive income"	Permitted, but not required.	Required.
Correction of errors	May either restate prior financial statements or include the cumulative effect in net profit and loss in the current financial statements.	Must restate prior financial statement.
Basis of property, plant, and equipment	May use either fair value or historical cost.	Generally required to use historical cost.
Revenue recognition guidance	Generally revenue recognition principles are consistent with U.S. GAAP but contain limited detailed or industry specific guidance.	More specific guidance exists on revenue recognition particularly relating to industry specific issues. In addition, public companies must follow more detailed guidance provided by the SEC.

GAAP rules are considerably more detailed with stricter interpretations than IFRS rules. Under GAAP, accountants have more guidance with respect to how to deal with financial statement transactions, whereas IFRS provides accountants more leeway to use their judgment and interpretation. In

some cases, if accountants are under the pressure to increase earnings, IFRS would appear to provide an easier pathway to earnings management. One way that accountants could manage earnings is through the application of rules relating to conservatism.

### 3. The significant role of accounting conservatism in U.S. GAAP and IFRS

Conservatism in U.S. GAAP will be examined first. FASB Concepts Statement No. 2 defines conservatism as "a prudent reaction to uncertainty to try to ensure that uncertainty and risks inherent in business situations are adequately considered." In other words, conservatism is "the accountant's tendency to require a higher degree of verification to recognize good news as gains than to recognize bad news as losses" (Basu, 1997). Thus, conservatism is a tendency to understate income rather than overstate income when dealing with ambiguous circumstances. For example, if there is a possibility that a firm may lose in a lawsuit, the firm would record this loss in its financial statements. However, if there is a possibility that a firm may win a lawsuit, the firm would not record the gain in its financial statements. A reason behind conservatism is that business practices have to deal with uncertainties on a day to day basis, requiring accountants to account for ambiguous situations with care.

According to Ross Watts in his paper "Conservatism in Accounting," conservatism cannot be used to describe the net change in income statement for any given period. In fact, he argues that "conservatism refers to the cumulative financial effects represented in the balance sheet and to income or earnings cumulated since the firm began operation" (Watts, 2003). To determine whether a firm is conservative or not, Watts believes we need to look at changes in net assets of a firm overtime. A conservative firm will have a "persistent understatement of net asset values." The understatement of net assets at the current period can "lead to overstatement of earnings in a future period by causing an understatement of future expenses," which is why we cannot overlook a firm's conservatism by only employing the net change in income statement to describe conservatism (Watts, 2003).

Although conservatism requires firms to verify profits or gains before recording them in their financial statements, it does not mean that firms can only recognize revenues once they receive cash; instead, conservatism requires firms to verify their cash flows (Watts, 2003). For instance, under accrual accounting, firms can recognize revenues once they have delivered goods and services to customers or fulfilled any obligations with the clients. Firms do not have to wait until they receive cash from customers to recognize revenues to be "conservative".

Conservatism benefits users of financial statements in multiple ways. In the paper "The Information Role of Conservatism", LaFond and Watts argue "conservative financial reporting is a governance mechanism that reduces the managers' ability to manipulate financial performance and



increases the firm's cash flows and value" (LaFond and Watts, 2007). The authors explain their argument by stating that managers have a tendency to influence firms' performance and stock prices during their tenure. This inappropriate use of time "deflects their efforts from increasing firm value, generating agency costs and reducing firm value even more." While helping firms prevent their managers from influencing financial performance, conservatism also helps "reduce information asymmetry between managers and outside investors", benefiting all financial statements' users (LaFond and Watts, 2007).

Although there are many studies on accounting conservatism in U.S. GAAP, few if any studies have been done to determine the impact of conservatism in IFRS. This is a new topic within the accounting field. Accounting researchers are still in the process of determining exactly what will be the IFRS rules. With the SEC considering the adoption IFRS in near future, it is important to examine IFRS from different perspectives in order to weigh the costs and benefits of adopting IFRS on conservatism.

#### 4. Sample selection, hypotheses, and descriptive statistics

##### 4.1 Hypotheses

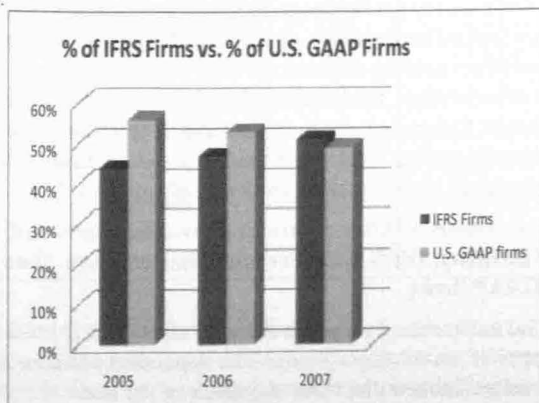
Using a 90% confidence level, the following hypotheses were tested:

- IFRS firms are more conservative than U.S. GAAP firms.
- IFRS firms have higher total assets than U.S. GAAP firms.
- Firms adopting IFRS have higher R&D intensity than firms adopting GAAP.
- After controlling for total assets and R&D intensity, IFRS firms are more conservative than U.S. GAAP firms.
- After controlling for the skewness of total assets and R&D intensity, IFRS is still responsible for a firm's conservatism.

##### 4.2 Sample selection

The sample includes firm-year observations from the Compustat Global Industrial/Commercial File from 2005 to 2007. All IFRS firms were obtained from Compustat Global. About half of the sample firms (48.6%) use IFRS, and the

Graph 3:



rest of the firms (51.4%) use U.S. GAAP. The chart below demonstrates how the percentage of IFRS firms and U.S. GAAP firms in the sample changed from 2005 to 2007.

According to the chart, the trend of firms adopting IFRS has increased consistently in three years. Specifically, the percentage of IFRS firms changed from 44% in 2005 to 51% in 2007, indicating the rising popularity of the IFRS accounting system.

The sample used in this analysis consisted of 1,625 firm-year observations, and measured 4 main factors: year end market value of equity in millions (MVE), total assets in millions, book value of equity divided by market value of equity (BTM), and research and development (R&D) divided by total revenue (RND\_REVENUE). Year-end market value of equity and total assets implies firm size. The bigger the firm, the more assets and equity it has. The BTM value measures how conservative the firm is; the lower value means the firm is more conservative. The RND\_REVENUE value measures how heavily a firm invests in its R&D. Under the same accounting system, a firm with high R&D intensity is often more conservative than a firm with less R&D intensity.

##### 4.3 Descriptive statistics

###### a. Hypothesis 1: Firms adopting IFRS are more conservative than firms adopting GAAP.

BTM values between IFRS firms and U.S. GAAP firms were compared using an independent t-test for two samples assuming unequal variances (determined through F-test comparisons of sample variances). Hypothesis 1 is true when BTM values for IFRS firms are significantly smaller than BTM values for U.S. GAAP firms at a 10% significance level ( $\alpha$ ). T-test result is shown in Table 2.

$$H_0 : BTM_{IFRS} - BTM_{GAAP} = 0$$

$$H_1 : BTM_{IFRS} - BTM_{GAAP} < 0$$

Table 2.

	IFRS	GAAP
Mean	0.817578233	0.888948905
Variance	0.503630364	0.628640377
Observations	789	836
Hypothesized Mean Difference	0	
t Stat	-1.914119837	
P(T<=t) one-tail	0.027890537	

Based on this t-test, the null hypothesis can be rejected. BTM values for IFRS firms are significantly smaller than BTM values for U.S. GAAP firms. However, BTM value is not a definitive factor in determining the conservatism of firms. Besides different accounting systems, other factors, such as firm size (total assets) or R&D intensity, can have an impact on the firms' conservatism. The next two hypotheses examine whether larger firm size (higher total assets) or higher R&D intensity can affect the conservatism of firms.

###### b. Hypothesis 2: IFRS firms have higher total assets (bigger size) than U.S. GAAP firms.

Values of total assets between IFRS firms and U.S. GAAP firms are compared using an independent t-test for two samples assuming unequal variances (determined through F-test comparisons of sample variances). Hypothesis 2 is true when total assets of IFRS firms are significantly larger than total assets of U.S. GAAP firms at a 10% significance level. Table 3 demonstrates the result of the t-test.

$$H_0 : \text{Total\_AssetsIFRS} - \text{Total\_AssetsGAAP} = 0$$

$$H_1 : \text{Total\_AssetsIFRS} - \text{Total\_AssetsGAAP} > 0$$

Table 3.

	IFRS	GAAP
Mean	29786.82293	2792934.859
Variance	205168289.5	2.18409E+15
Observations	789	836
Hypothesized Mean Difference	0	
t Stat	-1.709511734	
P(T<=t) one-tail	0.043863872	

Based on this t-test, the null hypothesis can be rejected. Total assets for IFRS firms are significantly smaller than the total assets for U.S. GAAP firms, which is the opposite result from the prior prediction. This result suggests that IFRS firms tend to be significantly smaller in size (own less total assets) than U.S. GAAP firms.

**c. Hypothesis 3: IFRS firms have higher R&D intensity than U.S. GAAP firms.**

R&D intensity between IFRS firms and U.S. GAAP firms are compared using an independent t-test for two samples assuming unequal variances (determined through F-test comparisons of sample variances). Hypothesis 3 is true when IFRS firms have significantly higher R&D intensity than U.S. GAAP firms. Table 4 illustrates the result of the t-test.

$$H_0 : \text{RND\_REVENUEIFRS} - \text{RND\_REVENUEGAAP} = 0$$

$$H_1 : \text{RND\_REVENUEIFRS} - \text{RND\_REVENUEGAAP} > 0$$

Table 4.

	IFRS	GAAP
Mean	0.014906459	0.007576989
Variance	0.00132021	0.000498483
Observations	789	836
Hypothesized Mean Difference	0	
t Stat	4.865230417	
P(T<=t) one-tail	6.41781E-07	

The result of this t-test suggests that IFRS firms invest more heavily in R&D compared to U.S. GAAP firms. That is, IFRS firms have a significantly higher ratio between R&D spending and total revenue.

A firm's R&D intensity can play a major role in determining the firm's degree of conservatism. For example, pharmaceutical companies have higher R&D intensity and are more conservative (smaller BTM value) than non-pharmaceutical firms, although they practice the same accounting system. To illustrate this fact, a simple regression was run to examine the relationship between R&D intensity

Table 5.

Regression Statistics					
Multiple R	0.055261701				
R Square	0.003053856				
	df	SS	MS	F	Significance F
Regression	1	2.821283296	2.82128	4.971590166	0.025903292
	Coefficients	Standard Error	t Stat	P-value	
Intercept	0.869679057	0.019920319	43.6579	4.8228E-276	
RND_REVENUE	-1.381441487	0.619562088	-2.22971	0.025903292	

and BTM value. The independent variable was R&D intensity and the dependent variable was BTM value. A negative relationship between two variables was expected. A negative value would mean that the higher a firm's R&D intensity, the lower its BTM value (more conservative). Table 5 illustrates the result of the regression test.

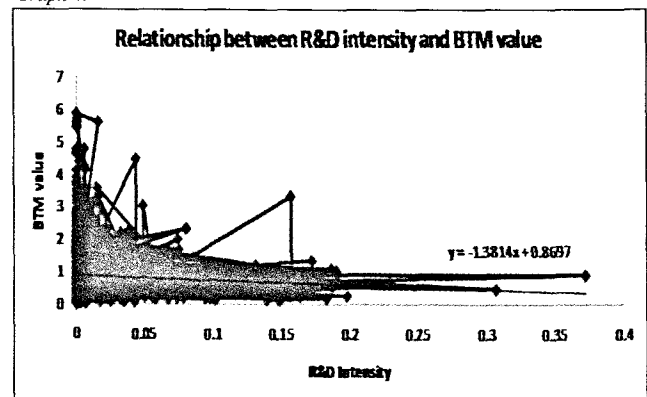
$$H_0 : \beta \text{RND\_REVENUE} = 0$$

$$H_1 : \beta \text{RND\_REVENUE} \neq 0$$

The equation of this regression is:  $\text{BTM} = 0.8696 - 1.3814 * \text{RND\_REVENUE}$

The small p-value ( $p = 0.0259$ ) implies that there is a significant linear relationship between R&D intensity and BTM value. The negative coefficient (-1.38144), together with the small p-value, suggests that there is a significant negative linear relationship between R&D intensity and BTM value. The graph below illustrates this linear relationship.

Graph 4.



The results of this regression test suggest that a firm's conservatism may not be due to different accounting systems, but may instead be the result of R&D intensity. To confirm if different accounting systems are truly accountable for a firm's conservatism, R&D intensity was controlled in the next hypothesis. Referring back to Hypothesis 2, IFRS firms have significantly smaller total assets than U.S. GAAP firms. Thus, firm size (total assets) was controlled in Hypothesis 4.

**d. Hypothesis 4: After controlling for total assets and R&D intensity, IFRS firms are more conservative than U.S. GAAP firms.**

The independent variables are total assets, R&D intensity, and types of accounting systems. The dependent variable is BTM value. Table 6 illustrates the result of the test.



Table 6.

Regression Statistics					
Multiple R	0.069331067				
R Square	0.004806797				
	df	SS	MS	F	Significance F
Regression	4	4.440725911	1.11018148	1.95615557	0.098767735
	Coefficients	Standard Error	t Stat	P-value	
Intercept	0.901104443	0.027568982	32.6854446	2.1E-180	
IFRS	-0.06691711	0.040006452	-1.67265791	0.09458775	
RND	0.496598215	1.381809185	0.35938263	0.71935571	
Total_assets	1.78662E-11	5.57925E-10	0.03202263	0.97445795	
RND_REVENUE	-1.61085571	1.167845878	-1.37933929	0.16798054	

$$H_0 : \beta_{IFRS} = \beta_{RND} * IFRS = \beta_{Total\ assets} = \beta_{RND\_REVENUE} = 0$$

$$H_1 : \text{At least one } \beta \neq 0$$

The equation of the regression is:  $BTM = 0.9011 - 0.0669 * IFRS - 0.4965 * RND + 1.786E-11 * Total\_assets - 1.6108 * RND\_REVENUE$ .

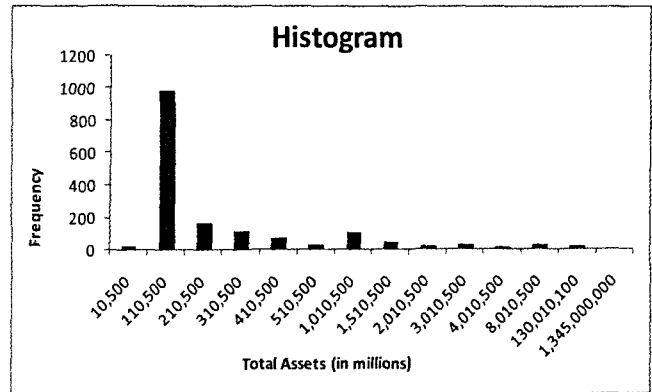
Based on this regression analysis, there is still a significant negative linear relationship between IFRS and a firm's conservatism after controlling for total assets and R&D intensity. The small p-value ( $p = 0.0974$ ) supports this conclusion. There is, however, no significant linear relationship between total assets and BTM value ( $p = 0.9744$ ). Lack of such a relationship can be due to the uneven distribution of total assets among firms. For example, some firms have significantly higher total assets than other firms do in the sample. In fact, the smallest total asset (in millions) of the sample was 10,014, while the largest total asset of the sample was 1,342,078,000. Table 7 and the histogram following illustrate the uneven distribution of total assets among sample firms.

Table 7.

Total Assets (in millions)	Frequency	% of total firms
10,500	19	1.17%
110,500	986	60.68%
210,500	162	9.97%
310,500	109	6.71%
410,500	68	4.18%
510,500	29	1.78%
1,010,500	100	6.15%
1,510,500	46	2.83%
2,010,500	19	1.17%
3,010,500	31	1.91%
4,010,500	12	0.74%
8,010,500	25	1.54%
130,010,100	18	1.11%
1,345,000,000	1	0.06%

Table 7 demonstrates the bin range of total assets (in millions). The frequency counts how many times total assets of sample firms are less or equal to the corresponding bin

Graph 5: Histogram:



number. For example, the frequency of 19 at bin range 10,500 means that there are 19 firms that have total assets less or equal to 10,500. The bin range and frequency are used to create the histogram in

According to Table 7 and Graph 5, a majority of sample firms (60.68%) have total assets (in millions) between 10,500 and 110,500. Very few firms (0.06%) have total assets greater than 130,010,100. The distribution of total assets is positively skewed, and this can distort the result of the multiple regressions in Hypothesis 4. Thus, to accurately determine whether different accounting systems are responsible for a firm's conservatism, the next hypothesis controlled for R&D intensity and the skewness of total assets. The skewness of total assets can be controlled by taking the log value of total assets.

Table 8.

Regression Statistics					
Multiple R	0.104501398				
R Square	0.010920542				
	df	SS	MS	F	Significance F
Regression	4	10.08886713	2.522216783	4.471652482	0.001350521
	Coefficients	Standard Error	t Stat	P-value	
Intercept	0.296030782	0.193175908	1.532441523	0.125608768	
IFRS	-0.216124046	0.061719199	-3.501731189	0.000474785	
RND*IFRS	0.491433851	1.377426484	0.356776828	0.721305349	
Log of total assets	0.059103886	0.018676611	3.164593732	0.001581855	
RND_REVENUE	-1.563245034	1.164195271	-1.342768755	0.17953497	

**e. Hypothesis 5: After controlling for R&D intensity and total assets, IFRS firms are more conservative than U.S. GAAP firms.**

The four independent variables of the regression were: IFRS, RND \* IFRS, log of total assets, and RND\_REVENUE. RND \* IFRS and log of total assets were two variables that control RND intensity and the skewness of total assets, respectively. The dependent variable was BTM value. The result of hypothesis 5 is shown in Table 8.

$$H_0 : \beta_{IFRS} = \beta_{RND} * IFRS = \beta_{Log\ of\ total\ assets} = \beta_{RND\_REVENUE} = 0$$

$$H_1 : \text{At least one } \beta \neq 0$$

The equation of the regression is:  $BTM = 0.296 - 0.2161 * IFRS + 0.4914 * (RND * IFRS) + 0.0591 * (\log \text{ of total assets}) - 1.563 * RND\_REVENUE$ .

There are two interesting findings in this regression analysis. First, a small p-value of 0.00047 shows different accounting systems are still accountable for a firm's conservatism after controlling for R&D intensity and the skewness of total assets. Specifically, firms adopting IFRS are still more conservative than firms adopting U.S. GAAP. Second, a significant positive relationship between log of total assets and BTM values ( $p = 0.00158$ ) implies that IFRS seems to be more conservative in accounting for total assets. This result is consistent with the final conclusion in Hypothesis 2. IFRS firms tend to be smaller, or have less total assets, than U.S. GAAP firms.

## 5. Conclusions

Inspired by an increasing popularity of IFRS and the possible adoption of IFRS in the U.S., this paper compared accounting conservatism practices within IFRS and U.S. GAAP. The sample included 1,625 firms, over half of which practice U.S. GAAP. To determine the conservatism of IFRS firms compared to U.S. GAAP firms, the following were tested: (1) differences between total assets, and R&D intensity for two groups, and (2) the relationship between methods of accounting and firms' conservatism when not controlling for any factors, when controlling for R&D intensity and total assets, and when controlling for R&D intensity and the skewness of total assets. Differences were analyzed using t-tests and relationships were analyzed using regression procedures.

Most of the initial hypotheses were confirmed. Firms using IFRS have smaller book-to-market values than firms adopting U.S. GAAP, implying that IFRS firms are more conservative than U.S. GAAP firms. IFRS firms also have smaller total assets (smaller size) than U.S. GAAP firms. In addition, after controlling for all factors that may affect firms' conservatism, such as R&D intensity, total assets, and skewness of total assets, IFRS firms are still more conservative than U.S. GAAP. This suggests that IFRS is responsible for a firm's conservatism.

This study is one of the first known comparisons of conservatism across IFRS and U.S. GAAP accounting practices. In previous research, it has been suggested that U.S. GAAP is more comprehensive and rules-based than IFRS, which is more principles-based. This has led to the assumption that IFRS could be more easily manipulated and accountants could use discretion in applying the principles, which would lead to a decrease in conservatism and an increase in overstatement of net income. In contrast, the current study has established that IFRS follows the rules of conservatism more closely than U.S. GAAP.

There are multiple elements that affect a firm's conservatism. Examination of some of these factors in the current study provided further credibility to the theory that

IFRS will be more conservative than U.S. GAAP. Hopefully, this study will provide a foundation for additional research related to the use of IFRS in the United States.

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#### Mentor Comments:

Honors thesis mentor Carole Shook places Hang Pham’s work in context by describing anticipated changes accounting principles and the questions raised by those changes with respect to conservatism in accounting. She emphasizes the originality of Hang’s work.

*Hang Pham’s honors thesis research explores the newest and potentially most change in accounting standards ever. She examined International ‘Financial Reporting Standards (IFRS) to determine if IFRS, or the current standards used in the United States, Generally Accepted Accounting Standards (GAAP), have more conservatism. There is no research like it published in the world. The topic is timely and her work is important for all companies adopting IFRS both in the U.S. and in the world.*

*IFRS is currently planned to be adopted in the U.S. in 2014, although non-U.S. companies who use IFRS and sell securities in the U. S. do not need to convert their financial statements to U.S. GAAP. IFRS is already being used in over one hundred countries and was adopted by the European Union in 2005. The reason for the change from GAAP to IFRS in the U.S. centers on the need for financial statements that*

*can be understood by investors from around the world non matter which country they are prepared in. Today’s world is a global marketplace and using different financial accounting standards in every country hinders the ability of companies to find investors and grow, which slows a worldwide economy.*

*GAAP is a rules based system. All accounting reporting rules are carefully spelled out. IFRS is a system based on judgment. That means that accountants and auditors have a wide latitude in choosing how to deal with specific accounting issues. This judgment opens the door for companies to manipulate financial records, which potentially leads to earnings management and fraudulent financial reporting on a worldwide level. Conservatism using GAAP requires a higher level of proof to record revenue (good news) than to record expenses (bad news). With IFRS there is currently no information on conservatism. Conservatism is potentially one of the main ways under which earnings management can occur using IFRS. Hang conducted detailed statistical analyses and controlled for multiple factors in her hypotheses. She was able to determine that despite judgment being used by IFRS, companies using IFRS appeared to be more conservative. This is a major and important discovery.*

*Since IFRS is such a new area, accounting researchers are just beginning to gather and analyze data on the effects. This makes Hang’s work completely innovative and distinctive. Her results will be among the first recognized works in this vital new area of accounting, especially in regards to the combination of IFRS and conservatism.*

*I was her primary thesis advisor. Due to the difficulties of collecting data, Dr. James Myers of the University of Arkansas was asked to be co-advisor. Dr. Myers had access through his editorial duties at a major accounting journal and connections to other accounting researchers to a database (Compustat Global) that is not available at the University of Arkansas. He assisted Hang in gathering the data that she used in her analysis.*

*Hang came up with this topic independently. Dr. Myers has begun to conduct research in IFRS and is an expert on the topic of conservatism in U. S. GAAP, and he provide some guidance as to articles related to conservatism and GAAP. However, his contributions apart from access to the database were minimal. The work and the ideas presented in this article belong exclusively to Hang.*

## ANALYSIS OF THE ACCASE MUTATION PROFILE OF ITALIAN RYEGRASS (*LOLIUM PERENNE* SSP. *MULTIFLORUM*) ACCESSIONS RESISTANT TO ACCASE INHIBITORS

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Faculty Mentor: Nilda Burgos

Department of Crop, Soil, and Environmental Sciences

### Abstract

*Lolium perenne ssp. multiflorum* (Italian ryegrass) resistant to ACCase inhibiting herbicides has been reported in many wheat producing counties across Arkansas. Resistance is believed to be the result of point mutations creating amino acid substitutions in the CT domain of the plastidic ACCase gene. This study explores the occurrence of mutations in the ACCase gene of ryegrass populations. Plant material was collected and DNA was extracted from 10 Arkansas ryegrass populations. Six of the populations were known to be resistant to the ACCase inhibitor diclofop-methyl, while the remaining four populations were known to be susceptible to diclofop-methyl. Two highly conserved regions of the plastidic ACCase gene known to contain mutations that confer resistance to ACCase inhibiting herbicides were then amplified and sequenced. Analysis of the sequences revealed that only 41% of the resistant populations expressed a mutation known to confer resistance. Several resistant populations of ryegrass did not contain any of the known mutations in their plastidic ACCase gene. This result means that either a mutation in a different region of the CT domain affects the affinity to ACCase inhibiting herbicides or the plants harbor a different mechanism of resistance. Further, in some resistant populations, not all plants within that population possessed a mutation known to cause resistance to ACCase inhibitors. This suggests that within a population, multiple mechanisms of resistance may exist. Further research is needed to determine the mechanism of resistance in diclofop-resistant plants that do not harbor mutations in the tested ACCase herbicide-binding domains.

### Introduction

The use of herbicides is the most effective and economical weed control method for large farms and is the principle method of weed control adopted by crop producers. Herbicides disrupt essential biological processes in plants, causing plant death. Plants that possess the heritable ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type are classified as resistant (Heap 2005). Currently there are 121 herbicide-resistant weed species in the United States, and this number is growing rapidly with the continued reliance on herbicides (Heap 2008). One class of herbicide-resistant weeds is those that are resistant to acetyl-CoA carboxylase (ACCase) inhibitors.

**The ACCase enzyme.** ACCase is a key enzyme in fatty acid biosynthesis and is one of the common targets of herbicide action. Higher plant ACCase is a multifunctional, biotinylated enzyme that catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA, the precursor to fatty acids as well as to secondary metabolites such as flavonoids (Evenson et al. 1994). In plants there are two isoforms of ACCase; plastidic and cytosolic. The plastidic ACCase is essential in the biosynthesis of primary fatty acids and the cytosolic ACCase is involved in the biosynthesis of long-chain fatty acids (Yu et al. 2007). The homomeric ACCase in the cytosol of nearly all plant species and the heteromeric ACCase in the chloroplasts of dicots are insensitive to ACCase inhibitors. In contrast, the plastidic homomeric ACCase in nearly all grass species is herbicide sensitive, and this is the basis for selective control of grass weeds by ACCase herbicides. In grasses, like *Lolium multiflorum* (Italian ryegrass), 80% of enzymatic activity is associated with the plastidic form of the enzyme (De Prado et al. 2000). All ACCase isoforms contain three catalytic domains: the biotin carboxyl carrier, the biotin carboxylase, and the carboxyl transferase (CT) domains. The CT domain of the plastidic homomeric ACCase is the primary target site for ACCase inhibitor herbicides (Yu et al. 2007).

**ACCase inhibitors.** Herbicides that inhibit ACCase are often termed graminicides as they only affect grass species with virtually no effect on dicotyledonous species (De Prado et al. 2000). Three chemically distinct classes of herbicides known to inhibit ACCase are aryloxyphenoxypropionates (AOPPs), cyclohexanediones (CHDs), and a phenylpyrazolin class of herbicide called pinoxaden (Yu et al. 2007). It has long been reported that both AOPPs and CHDs inhibit the transfer of carbon dioxide to acetyl-CoA that is catalyzed by the CT subunit (Délye et al. 2002). This effectively kills sensitive plants by shutting down fatty acid biosynthesis (Zhang et al. 2003). Previous studies have shown that graminicides are reversible, linear, noncompetitive inhibitors of ACCase in susceptible grasses and that the carboxyltransferase reaction is more sensitive to herbicide inhibition than is the biotin carboxylase reaction (Evenson et al. 1994).

**Evolution of resistance.** Today there are more than 200 herbicides used worldwide with 28 different modes of action (Vencill et al. 2002). The use of herbicides over time has resulted in the selection of weeds resistant to many

different classes of herbicides, with detrimental effects on weed management practices (Kaundun and Windass 2006). Extremely rare genetic mutations occur in nature; these impart tolerance to an herbicide. Repeated and widespread application of the same herbicide exerts a large-scale, high selection pressure on the weed population, selecting for those rare mutation events. Once one or a few individuals are selected and favored to reproduce in this system, tolerant individuals proliferate, giving rise to a resistant population. Thus, the widespread adoption of herbicides for grass weed control throughout the world has resulted in the appearance of numerous resistant weed populations.

The first report of resistance to ACCase-inhibiting herbicides came from Australia in 1982 and involved a *Lolium rigidum* (annual ryegrass) population resistant to diclofop-methyl (Bravin et al. 2001). *Lolium* is a genus composed of multiple species, mostly annual and mostly cross-pollinated. *Lolium multiflorum* is an annual, outcrossing species. Thus, genetic variability within and among populations is high, and the probability of selecting rare mutation events that could impart resistance to herbicides is also higher relative to self-pollinated species. Today, resistance to herbicides and other pesticides has become the number one concern in crop production.

**Mechanisms of resistance.** Resistance to ACCase-inhibiting herbicides may be conferred by one or more of five mechanisms. The first of these is a membrane mutation resulting in the ability to repolarize the membrane after herbicide-induced depolarization. In the SLR 31 biotype of *Lolium rigidum*, resistance to diclofop has been correlated to the ability of plants to recover from membrane depolarization events (Holtum et al. 1991). The second is a difference in the absorption and translocation of the herbicide between resistant and susceptible species. Biotypes of *Lolium rigidum* in Chile and Mississippi have expressed resistance to glyphosate partly due to reduced absorption and translocation of glyphosate (Michitte et al. 2007; Nandula et al. 2008). While glyphosate is not an ACCase-inhibiting herbicide, the same mechanism of resistance could confer resistance to ACCase inhibitors.

The third mechanism is the ability of resistant plants to metabolize or detoxify the herbicide. Populations of *Lolium multiflorum* in the United Kingdom have been found to confer resistance to ACCase-inhibiting herbicides through an enhanced rate of metabolism (Cocker et al. 2001). The fourth mechanism is an induction of ACCase to compensate for the inactivated enzyme. Biotypes of *Sorghum halepense* (johnsongrass) resistant to sethoxydim have been reported to have an increased production of ACCase (Bradley et al. 2001). Finally, resistance to ACCase-inhibiting herbicides may be due to an alteration in the target site. Reduced enzyme sensitivity to the inhibitor is an indication of this (Kuk et al. 2000).

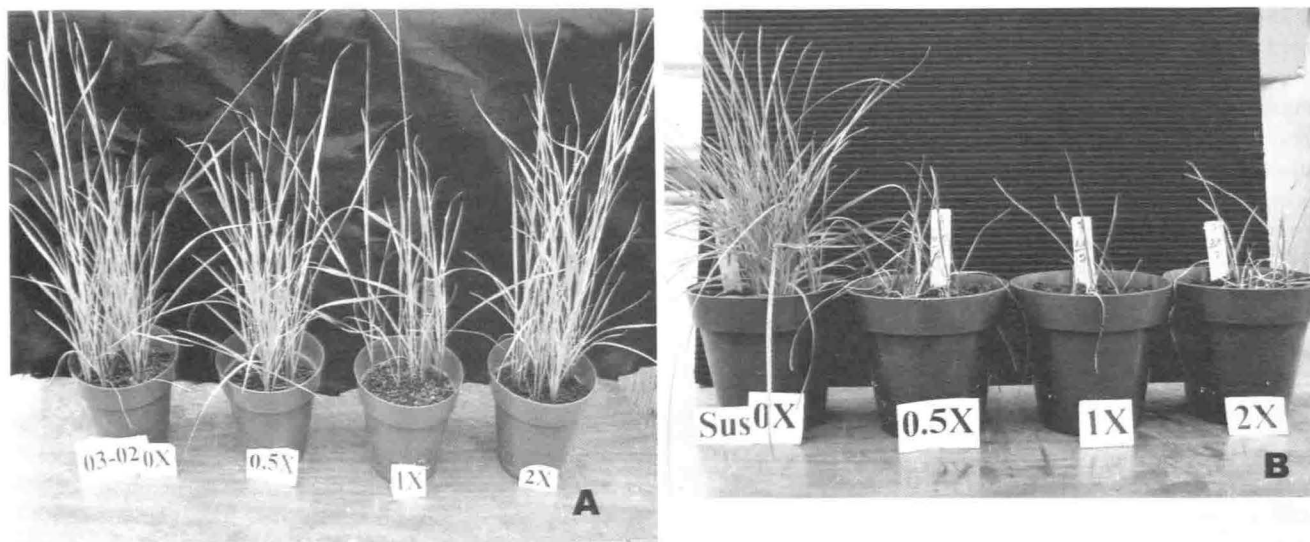
Documented cases of resistance to ACCase inhibitors due to enhanced metabolism, absorption, and translocation are few. These mechanisms are believed to confer generally low

<https://scholarworks.uark.edu/inquiry/vol10/iss1/1>

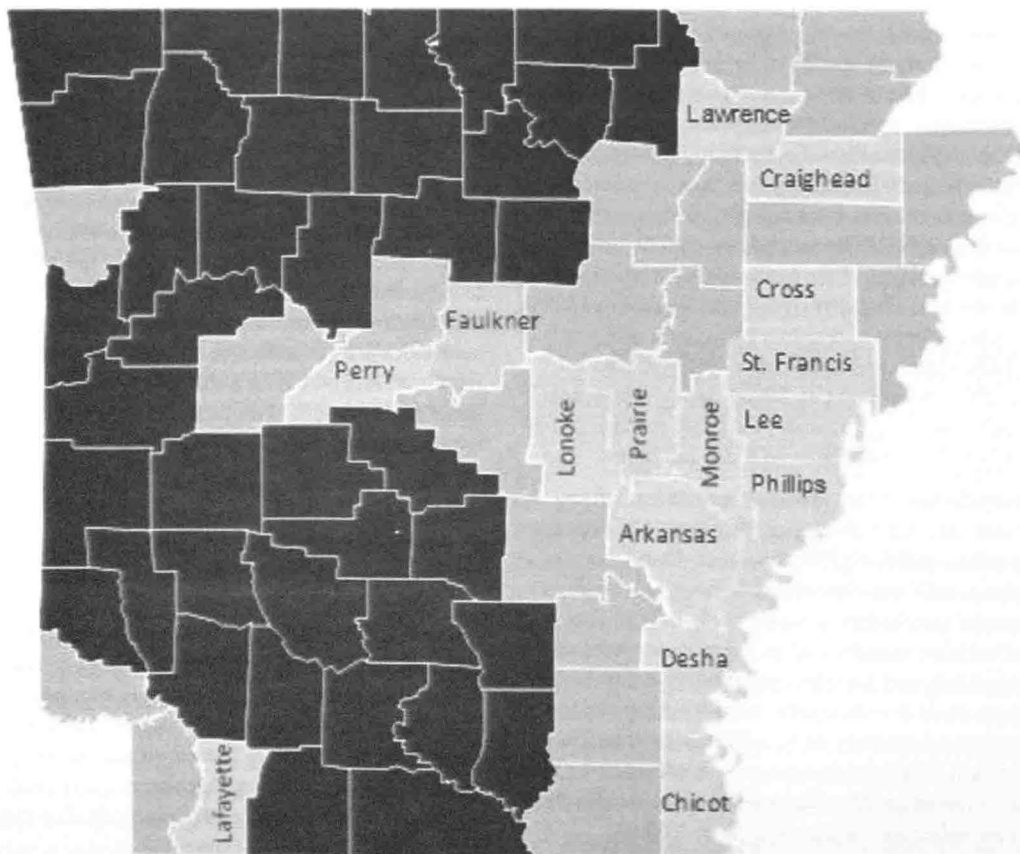
resistance levels (Kuk et al. 2000). Most cases of resistance to ACCase inhibitors are due to target site mutations. Thus far, in the CT domain of the plastidic ACCase gene, six distinct amino acid substitutions that individually endow resistance to certain ACCase herbicides have been characterized in different *Lolium* spp. These mutations (Ile<sub>1781</sub>, Trp<sub>2027</sub>, Ile<sub>2041</sub>, Asp<sub>2078</sub>, and Gly<sub>2096</sub>) confer resistance to one or more of nine selective grass herbicides (Délye et al. 2002; Délye 2008). Cross resistance to multiple herbicides is common in herbicide-resistant species. However, as of 2007, experiments showed that ryegrass biotypes resistant to ACCase-inhibiting herbicides in Arkansas were not resistant to other herbicides with different modes of action (Kuk et al. 2000; Kuk et al. 2008).

**Significance of this research.** Ryegrass is a major weed problem in wheat production. Ryegrass competes with wheat for nutrients, inhibits tillering, causes lodging, and interferes with harvesting, causing up to 90% yield losses for growers (Crooks et al. 2003). The evolution of resistant ryegrass makes weed management in wheat production difficult and expensive (Fig. 1). The most predominant species of *Lolium* in Arkansas is *L. perenne* ssp. *multiflorum*. Widespread ryegrass resistance to ACCase-inhibiting herbicides has been documented in Arkansas (Fig. 2). As of 2007, there were about 100 cases of confirmed diclofop-resistant ryegrass populations in Arkansas and the adjacent wheat producing areas of Missouri and Louisiana (Kuk et al. 2008). It is hypothesized that resistance to ACCase inhibitors in Arkansas ryegrass populations is due to mutations in the herbicide-binding domains of ACCase. Thus, research on the occurrence of mutations in the ACCase gene of ryegrass populations was conducted. Sequencing the ACCase gene of herbicide-resistant ryegrass exhibiting different resistance patterns to various graminicides can potentially reveal specific mutations that underlie the whole-plant response to different herbicides. Researchers and extension agents could then use this information in determining which alternative herbicides are still effective for certain ryegrass populations. However, this would be a short-term cure. In the long term, sustainable integrated management practices will have to be implemented to curb the evolution of resistant populations.

**Goals and Objectives.** This research was designed to: (1) sequence the herbicide-binding domains of the carboxytransferase (CT) subunit of the ACCase enzyme; (2) assemble and analyze gene sequences for nucleotide polymorphisms; and (3) translate nucleotide sequences into amino acid sequences to identify amino acid mutations and correlate this with whole-plant responses to specific herbicides. The overall goals of the project were to relate mutation patterns in the herbicide-binding site of the ACCase enzyme of Italian ryegrass to its resistance pattern to various ACCase inhibitors and to identify possible molecular markers useful for predicting herbicide cross-resistance behavior in Italian ryegrass populations.



**Figure 1.** Ryegrass resistant to ACCase-inhibiting herbicides represents a major problem for wheat farmers. (A) shows the growth of resistant *Lolium multiflorum* plants at 0 to 2X rates of diclofop. (B) shows the growth of susceptible *Lolium multiflorum* plants at the same rates of diclofop. 1X is the recommended rate of application and is equal to 1.12 kilograms per hectare of active ingredient.



**Figure 2.** Map of wheat-producing counties in Arkansas. Wheat-producing counties with identified cases of resistant ryegrass are in cyan. Wheat producing counties with no known cases of resistance are in salmon. Counties in black do not produce

## Materials and Methods

**Plant material.** Ten ryegrass populations were used in this experiment. Six of the populations, 01-1, 04-2, 04-3, 05-7, 98-2, and 98-18, were known to be resistant to the ACCase inhibitor diclofop. The other four, 05-1, 05-2, Missouri (MO), and Texas (TX), were known to be susceptible. Seedlings were grown in the greenhouse and leaves from 4 seedlings per population were harvested. The tissues from each plant were then wrapped separately in aluminum foil and stored at  $-80^{\circ}\text{C}$  until processed.

**Extraction of genomic DNA.** Genomic DNA was extracted using a hexadecyltrimethyl-ammonium bromide (CTAB) protocol by Doyle and Doyle (1987) with slight modifications. Briefly, 0.1 g of leaf tissue from each plant was ground to a fine powder in liquid nitrogen, transferred to 1.5-mL micro-centrifuge tubes and suspended in 500  $\mu\text{L}$  of CTAB extraction buffer (100 mM Tris-HCl [pH 8.0], 20 mM EDTA [pH 8.0], 2 M NaCl, 2% CTAB, 2% PVP-40, 1 mM phenanthroline, and 0.3%  $\beta$ -mercaptoethanol). The aqueous extracts were extracted once with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). Total nucleic acids were precipitated from the supernatant by addition of an equal volume of isopropanol. The DNA pellet was then washed with absolute alcohol, air-dried, and resuspended in 30  $\mu\text{L}$  of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). The genomic DNA was quantified by fluorometry, diluted to 50 ng  $\mu\text{L}^{-1}$  with sterile deionized water, and immediately used as a template for the amplification of the *ACCase* gene by polymerase chain reaction (PCR).

**Amplification of the CT domain of *ACCase*.** Two primer pairs, ACcp1/ACcp1R and ACcp4/ACcp2R, were used to amplify two regions of the plastidic CT subunit of the *ACCase* gene where known mutations causing resistance to ACCase inhibitors occur (Delye and Michel 2005). These are universal primers that have been tested on 20+ grass species. These primers produce fragments of the plastidic ACCase without contamination from the cytosolic ACCase. There are two obstacles to molecular studies of grass plastidic ACCase. First, the gene itself is large ( $>12,000$  bp) with a complex intron-exon structure. Second, there are two highly similar homomeric ACCase isoforms, and thus two highly similar *ACCase* genes in grasses. While one gene encodes the plastidic ACCase isoform that is highly sensitive to AOPPs and CHDs, the other encodes a cytosolic ACCase isoform that is tolerant to ACCase inhibitors (Delye and Michael 2005).

PCR amplifications were done in 20- $\mu\text{L}$  reactions containing 4  $\mu\text{L}$  5x buffer, 1  $\mu\text{L}$  2 mM  $\text{MgCl}_2$ , 1  $\mu\text{L}$  0.8 mM dNTPs, 2  $\mu\text{L}$  of each 0.2  $\mu\text{M}$  primer, 0.2  $\mu\text{L}$  of Promega Taq DNA Polymerase, and 50 ng of DNA. The final volume was then adjusted to 20  $\mu\text{L}$  using sterile deionized water (Zhang and Powles 2006). The cycling program consisted of one initial denaturation step of 30 s at  $95^{\circ}\text{C}$ , followed by 37 cycles of 10 s at  $95^{\circ}\text{C}$ , 15 s at the specific annealing temperature and 45 s at  $72^{\circ}\text{C}$ , followed by a final extension step of 10 min at

$72^{\circ}\text{C}$ . The amplicons produced for fragment 1 were purified and sequenced on both strands using the ACcp1 and ACcp1R primers. The amplicons produced for fragment 2 were purified and sequenced on both strands using the ACcp4 and ACcp2R primers (Delye and Michel 2005).

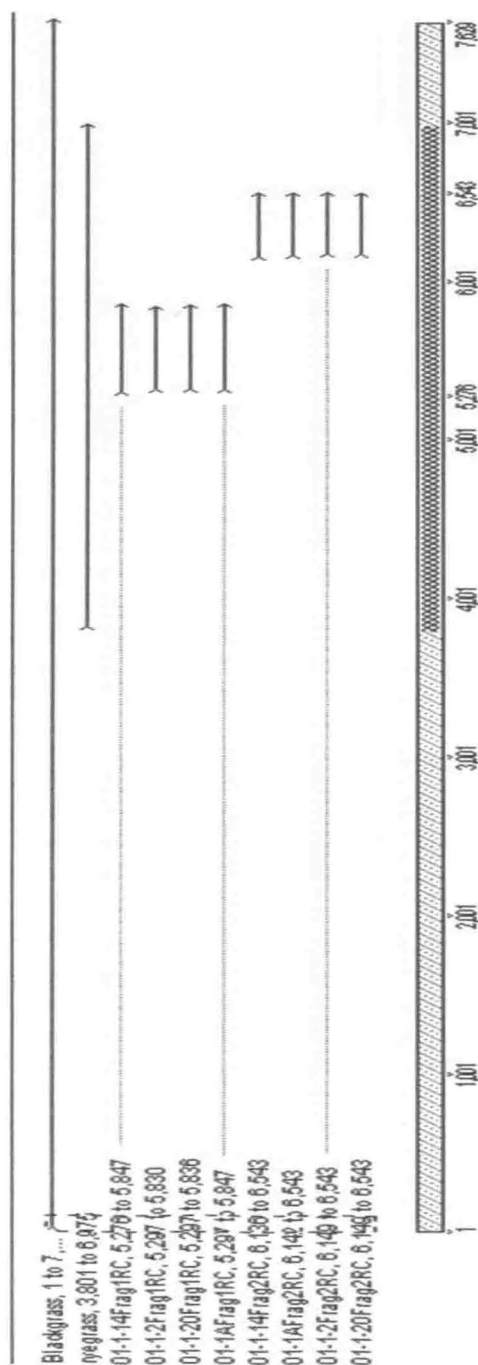
**Sequence analysis.** Sequences obtained were aligned and analyzed for polymorphisms using BioEdit and Sequencher software. Multiple alignments were then done using ClustalW. Finally, amino acid mutations were summarized in relation to the whole-plant response of each population to ACCase herbicides.

## Results and Discussion

The primer pair ACcp1/ACcp1R amplified the ryegrass nucleotide sequence creating fragment 1, while ACcp4/ACcp2R created fragment 2. Using *Alopecurus myosuroides* (blackgrass) as a reference sequence, fragment 1 amplified a region from base 5100 to 5660, while fragment 2 amplified a region from 5940 to 6360 (Fig. 3). Variation was present in the nucleotide sequences of both fragments within and across populations. The average percent nucleotide identity of fragment 1 between all populations was 99.467%. Fragment 2 had an average percent nucleotide identity of 98.7% (Table 1). The translated sequences fell between amino acid positions 1708 to 1886 and 1985 to 2118, respectively. The amino acid alignment revealed several mutations that are known to confer resistance in *Lolium* spp. Fragment 1 from all 10 populations did not contain any amino acid mutations known to lead to resistance, but mutations conferring resistance were found in fragment 2 in 41% of resistant populations sequenced.

At position 2027, all plants from the resistant 04-2 population encoded a cysteine rather than tryptophan (Fig. 5). This is a mutation that is known to cause resistance in *Alopecurus myosuroides* to clodinafop, diclofop, and haloxyfop. While this is not a mutation known to confer resistance to ACCase inhibitors in *Lolium* spp., some mutations in blackgrass also confer resistance in ryegrass, leading to the possibility that this mutation could cause resistance in *Lolium* spp. as well. Further experiments are needed to verify this. At position 2041, plants from resistant populations 98-18-8, 04-3-7, and 04-3-9 contained the mutation isoleucine to asparagine (Fig. 5). This mutation is known to confer broad resistance in *Lolium* spp. to the ACCase herbicides clodinafop, diclofop, and haloxyfop (Delye 2008). Also at position 2041, two plants in the diclofop-resistant population 98-2 contained an isoleucine to valine mutation (Fig. 5). This mutation also causes resistance to haloxyfop, but unlike the Ile<sub>2041</sub>Asn mutation, an Ile<sub>2041</sub>Val mutation is not known to cause resistance to diclofop or clodinafop (Delye 2008). Two of the resistant populations of ryegrass examined did not contain any mutations in their plastidic *ACCase* gene known to confer resistance to ACCase inhibitors. This means that either the plants contain a mutation in a different region of the CT subunit, which affected affinity to ACCase herbicides, or these plants harbor a different mechanism of resistance as discussed previously.





**Figure 3.** Alignment of the amplified *Lolium multiflorum* fragments 1 and 2 versus the *Alopecurus myosuroides* ACCase gene (AJ310767.1) and the partial *Lolium rigidum* ACCase gene (AY995233.1). Fragment 1 aligned with *Alopecurus myosuroides* from base 5100 to 5660, while fragment 2 aligned from base 5940 to 6360

Further, the fact that in several resistant populations, at least one but not all plants harbored a mutation known to cause resistance to ACCase inhibitors also suggests that within one population, multiple mechanisms of resistance may exist.

Accession Code	% Nucleotide Identity	
	Fragment 1	Fragment 2
01-1	99.2	97.7
04-2	99.5	99.5
04-3	99.3	98.7
05-1	100.0	98.2
05-2	99.5	98.3
05-7	99.3	98.3
98-2	99.3	99.2
98-18	99.5	98.7
MO	99.5	99.0
TX	99.5	99.5
<b>Overall Mean</b>	<b>99.5</b>	<b>98.7</b>

The occurrence of multiple mechanisms of resistance within a population has been documented in *Lolium rigidum* biotypes from Australia (Tardif and Powles 1994). This could be due to the mating behavior of *Lolium* spp. and its intrinsic high genetic diversity. Herbicide treatment of large populations results in the survival of individuals that express any resistance mechanism conferring the ability to withstand herbicide at the rate used. With obligate cross-pollinated species, there is gene flow among the survivors resulting in exchange of different resistance genes and their accumulation in the next generation (Tardif and Powles 1994). It appears that the same event is occurring in ryegrass populations in Arkansas. Further research is needed to determine the mechanism of resistance in the diclofop-resistant plants tested that did not harbor mutations in their ACCase herbicide-binding domains.

It is concluded that in Arkansas, at least for some ryegrass populations, target site mutation is causing broad resistance to various ACCase inhibitors. These mutations, however, do not confer resistance to acetolactate synthase (ALS) inhibitors, which are also used for ryegrass management in wheat. Therefore, a short-term management strategy for ACCase-resistant ryegrass could still utilize an alternative herbicide mode of action such as the ALS inhibitors. The potential presence of multiple mechanisms of resistance within populations or different mechanisms of resistance between populations entails diversification of cultural management practices so that weed management and wheat production will become sustainable.

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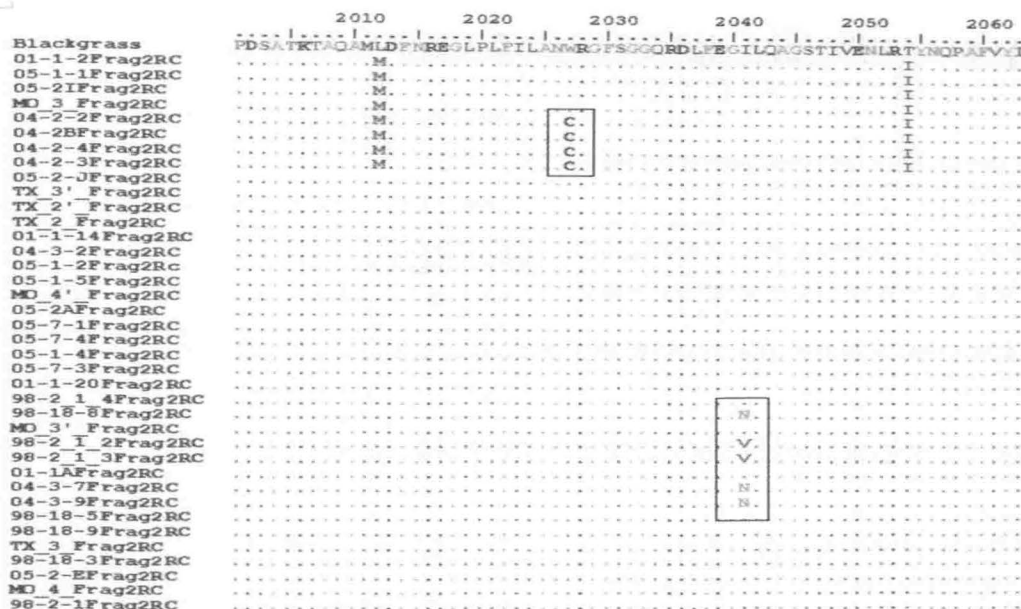


Figure 5. Ile<sup>204I</sup>, Asn<sup>204I</sup> and Ile<sup>204I</sup> Val are known to confer resistance in ryegrass to various ACCase inhibitors. Trp<sup>207Cys</sup> is known to cause resistance in *Alopecurus myosuroides* to ACCase inhibitors and will likely confer resistance in *Lolium* spp.

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- fuel, and fiber. Extensive use of herbicides has resulted in the evolution of resistance in some weed species and now there are more than 200 herbicide-resistant weed species worldwide, with about 130 cases in the US. As for ryegrass, it is a major weed problem in wheat-producing states and has evolved resistance to ALS, ACCase, and EPSPS inhibitor herbicides. Knowing the resistance pattern of ryegrass is important because growers need this information to determine which herbicides are still effective for the population in his field. This is a critical planning tool. In Arkansas, resistance to ACCase inhibitors is widespread. Most cases of resistance are due to genetic mutations in the herbicide target site and the resistance pattern corresponds to the type of mutations; therefore, Thomas' research was done to determine which mutations exist in the resistant ryegrass populations. ACCase mutation profiles in ryegrass are not found in the literature. Thomas' research provides basic information for future research on ACCase mutation profiling of this economically important weed, which will eventually be a decision tool for wheat growers, researchers, and the chemical industry in developing alternative, sustainable management strategies for ryegrass in wheat.

#### Mentor Comments:

Nilda R. Burgos highlights how Thomas Stark's work provides a foundation for building a decision tool for future work with herbicides and other chemicals that develop resistance over time.. She writes:

*I am writing this letter in support of the publication of the honors thesis of Thomas Stark entitled "Analysis of the ACCase mutation profile of Italian ryegrass (Lolium perenne ssp. multiflorum) accessions resistant to ACCase inhibitors" in the Inquiry Journal. Thomas was one of seven undergraduate honors students who applied for research mentorship in my laboratory in the fall of 2007, and one of two students I chose. He selected this research out of several alternative topics, all of which fit into the general area of my research program in weed physiology, biology, ecology, and molecular biology. Thomas received a full undergraduate research grant from the honors college for his thesis. My Ph.D. graduate students and I trained Thomas so he can conduct this research by himself. Beyond the initiation part, and assistance in sequence analysis, he did the rest of the work. Thomas' research is of great importance because it provides information about a critical issue in crop production, which is resistance to herbicides. The evolution of resistance to chemicals is not unique to agriculture; it happened first, and continues to be an issue, in the field of medicine. So this research has broader application for Thomas' education. In agriculture, pesticides (including herbicides) are widely used because it allows crop production in huge tracts of land to meet our needs for food,*